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Ministero dell'Università e della Ricerca

Italiadomani
PIANO NAZIONALE DI RIPRESA E RESILIENZA

HEAL ITALIA ROADSHOW
TECNOLOGIE E INNOVAZIONE
INNOVATION ON THE ROAD

PRIMO CONVEGNO NAZIONALE DEL CENTRO DI MEDICINA DI PRECISIONE — HEAL ITALIA PER LE MALATTIE RARE

www.ancona.centridimedicinadiprecisione.it

Responsabile scientifico
Prof. **Gianluca Moroncini**

UnivPM — Ancona
Aula Montessori
Facoltà di Medicina
e Chirurgia

venerdì 28 febbraio
14:30 → 18:30
sabato 1 marzo
09:00 → 13:00

Progetto "Health Extended ALliance for Innovative Therapies, Advanced Lab-research, and Integrated Approaches of Precision Medicine (HEAL ITALIA) Codice PE00000019. CUP I33C22006900006 – finanziato dal PNRR M4C2 I1.3 – DD MUR 341 del 15/03/2022

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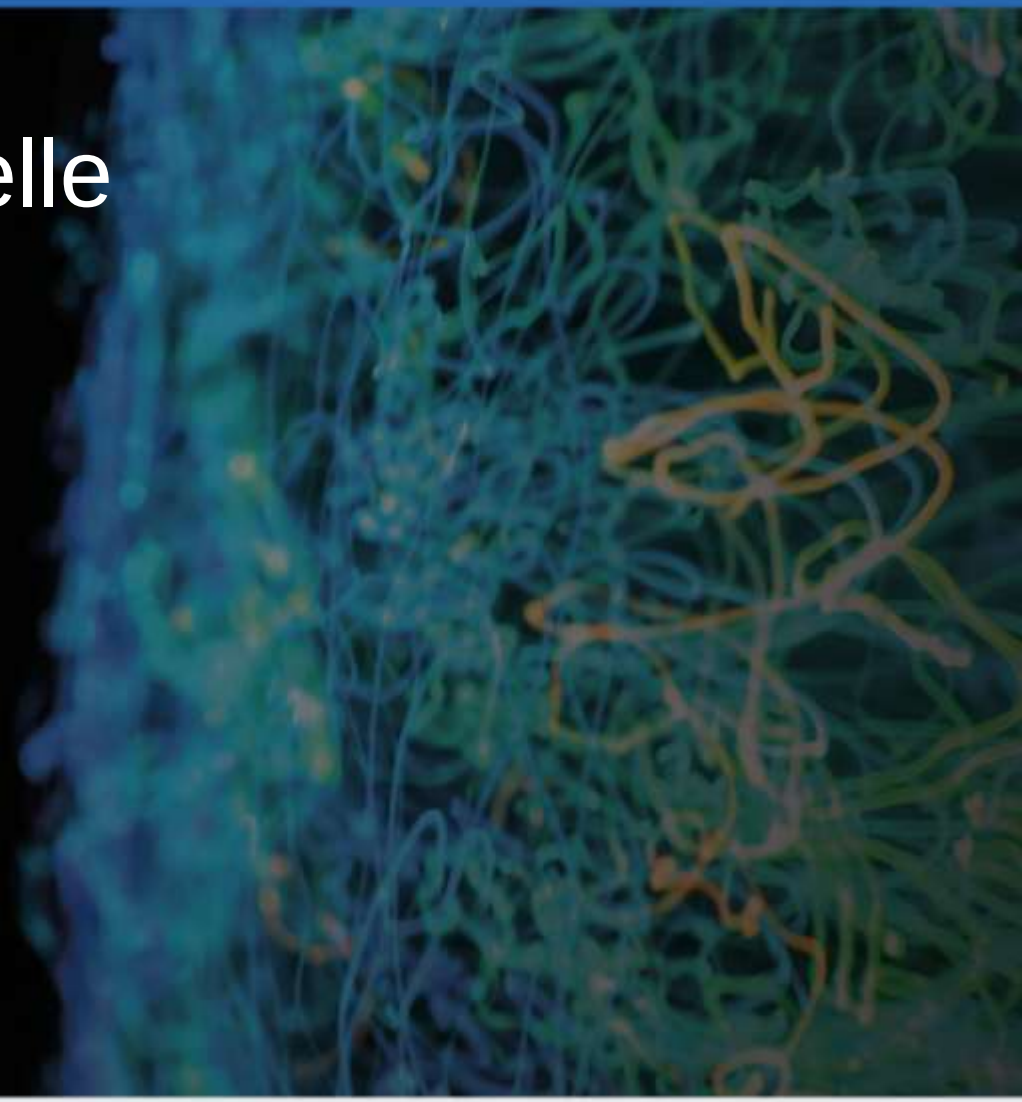
Il ruolo della Genetica Medica nelle malattie rare



Marco Seri

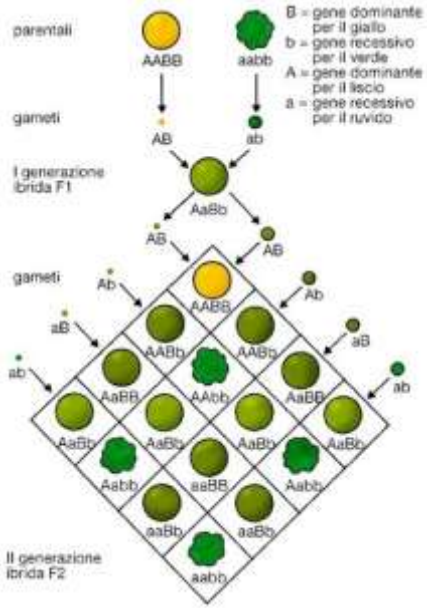
Direttore Scientifico IRCCS Azienda Ospedaliero-Universitaria di Bologna, Policlinico di Sant'Orsola

- La rivoluzione dell'era Genomica

- L'effetto del progetto genoma umano a livello clinico: l'approccio allo studio delle malattie genetiche rare tramite esoma

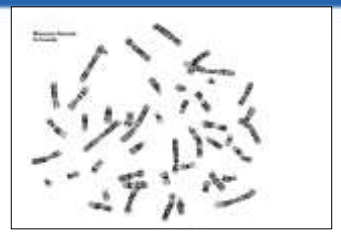
- L'utilizzo del genoma nei casi ancora non diagnosticati dopo esoma

La Genetica Medica 200 anni dopo Mendel



Gregor Johann *Mendel* (Hynčice, 20 luglio 1822 – Brno, 6 gennaio 1884)

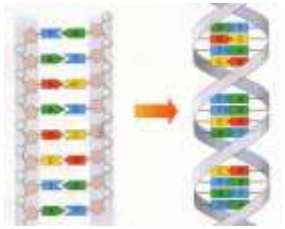
1956 description of the correct chromosome number in humans



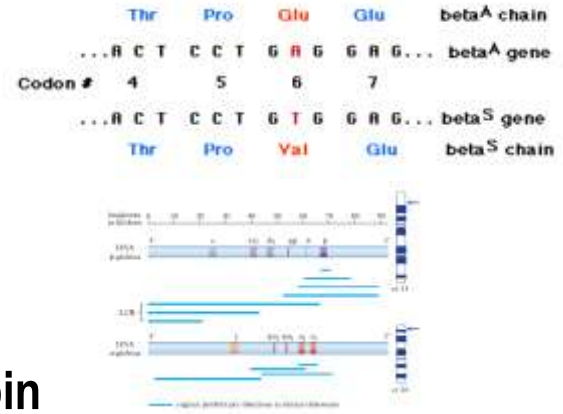
1959 discovery of a chromosome change associated with a clinical disorder (Down s.)

1902 concept of inborn errors of metabolism (alkaptonuria)

1953 structure of DNA



1957 single amino acid difference identified in the “sickle” hemoglobin protein



1966 “cracking” of the genetic code

1977 first human genes to be cloned: chorionic somatomammotropin, α- and β-globin

La rivoluzione del Progetto Genoma Umano

- 1985 – Proposto
- 1986 - 89 - Discusso, dibattuto e pianificato
- Oct. 1, 1990 – Data ufficiale di inizio progetto
- Sept. 30, 2005 – Data presunta di completamento del progetto
- ma…….


PRESIDENT OF THE UNITED

MENDELIAN INHERITANCE IN MAN (MIM)

1^ edizione 1966: 1500 voci – oggi: >26.000

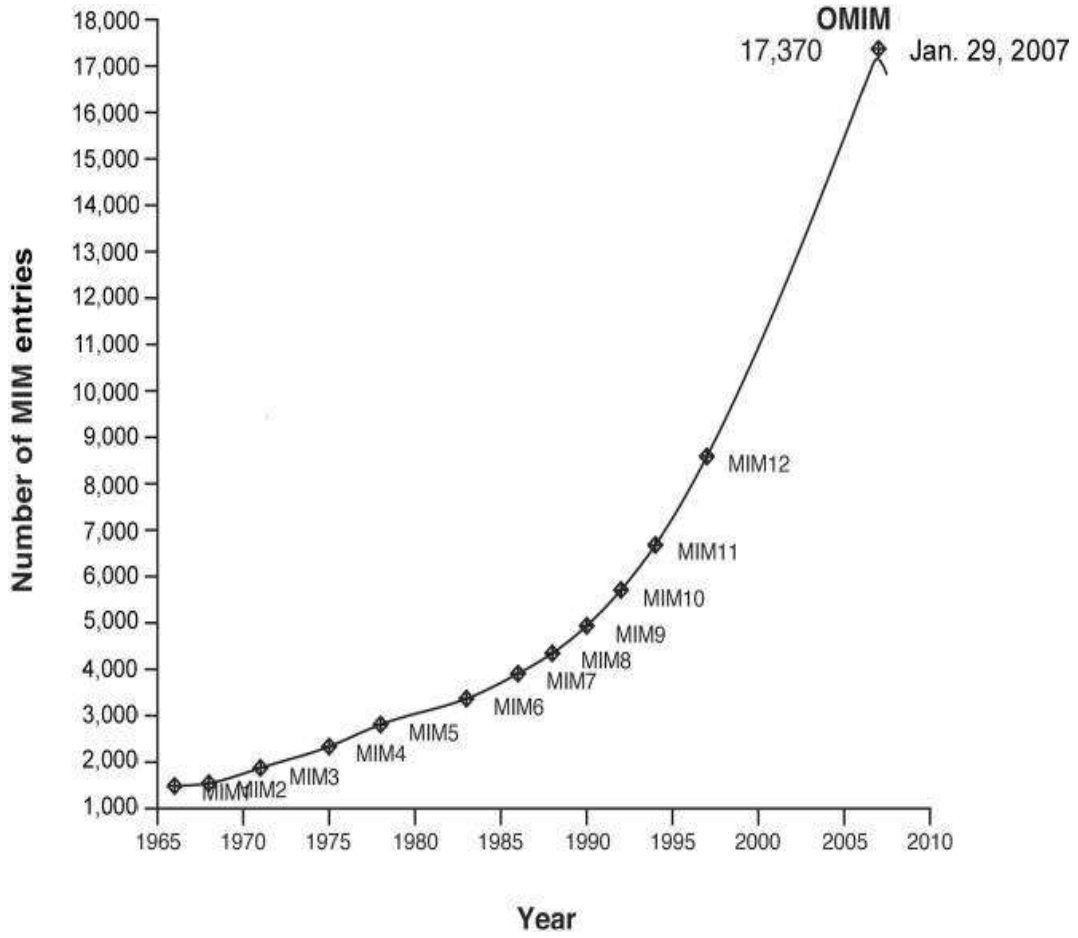
Dal 1998 solo in formato elettronico: On-lineMIM

http://www.ncbi.nlm.nih.gov/omim

OMIM

OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily. OMIM is authored and edited at the McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University School of Medicine, under the direction of Dr. Ada Hamosh. Its official home is omim.org.

Un incremento esponenziale di informazioni



OMIM Entry Statistics

Number of Entries in OMIM (Updated February 27th, 2025) :

MIM Number Prefix	Autosomal	X Linked	Y Linked	Mitochondrial	Totals
Gene description *	16,617	786	51	37	17,491
Gene and phenotype, combined +	14	0	0	0	14
Phenotype description, molecular basis known #	6,526	393	5	35	6,959
Phenotype description or locus, molecular basis unknown %	1,386	110	4	0	1,500
Other, mainly phenotypes with suspected mendelian basis	1,628	99	3	1	1,731
Totals	26,171	1,388	63	73	27,695

OMIM Morbid Map Scorecard (Updated February 27th, 2025) :

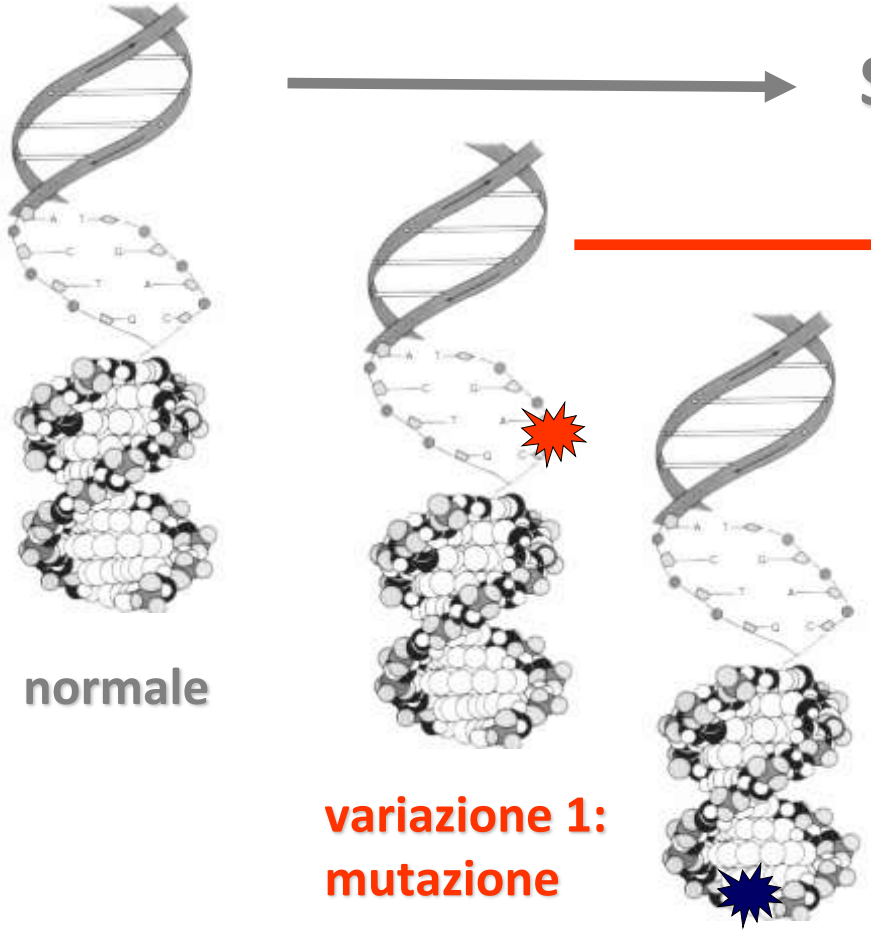
Total number of phenotypes* for which the molecular basis is known	7,601
Total number of genes with phenotype-causing mutation	4,966

* Phenotypes include (1) single-gene mendelian disorders and traits; (2) susceptibilities to cancer and complex disease (e.g., BRCA1 and familial breast-ovarian cancer susceptibility, 113705.0001, and CFH and macular degeneration, 134370.0008); (3) variations that lead to abnormal but benign laboratory test values ("nondiseases") and blood groups (e.g., lactate dehydrogenase B deficiency, 150100.0001 and ABO blood group system, 110300.0001); and (4) select somatic cell genetic disease (e.g., GNAS and McCune-Albright syndrome, 139320.0008 and IDH1 and glioblastoma multiforme, 147700.0001.)

Distribution of Phenotypes across Genes (Updated February 27th, 2025) :

Number of genes with 1 phenotype	3,480
Number of genes with 2 phenotypes	903
Number of genes with 3 phenotypes	330
Number of genes with 4+ phenotypes	253

Geni e malattie: una nuova dimensione



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nature Vol 452|17 April 2008|doi:10.1038/nature06884

LETTERS

The complete genome of an individual by massively parallel DNA sequencing

Sequenziamento personalizzato

HUMAN GENETICS

Dr Watson's base pairs

Maynard V. Olson

The application of new technology to sequence the genome of an individual yields few biological insights. Nonetheless, the feat heralds an era of 'personal genomics' based on cheap sequencing.

This issue of *Nature* contains a paper that is, in a curious way, a sequel to one published 55 years ago — the description by James Watson and Francis Crick[1] of the double-helical structure of DNA. At the information-carrying core of this beautiful structure, with its far-reaching implications for biology and medicine, are the base pairs that Watson discovered by fitting together cardboard cut-outs of the bases adenine, thymine, guanine and cytosine. Now, on page 872, Wheeler *et al.*[2] describe the use of massively parallel DNA sequencing to determine the order of the base pairs in Watson's own genome. This achievement is a technical *tour de force* that points towards routine use of whole-genome sequencing as a research tool in human genetics. Given the choice of James Watson as an identified research subject, the paper is also a conspicuous effort to publicize the arrival of the era of personal genomics and the willingness of a famous geneticist to put his genome sequence in the public domain.

Technically, the paper's interest stems from its reliance on a DNA-sequencing platform that differs greatly from the one used during the first great era of genome sequencing, which culminated in the Human Genome Project (HGP). In the HGP platform, each kilobase-pair fragment of genomic DNA was captured


James Watson decoded.

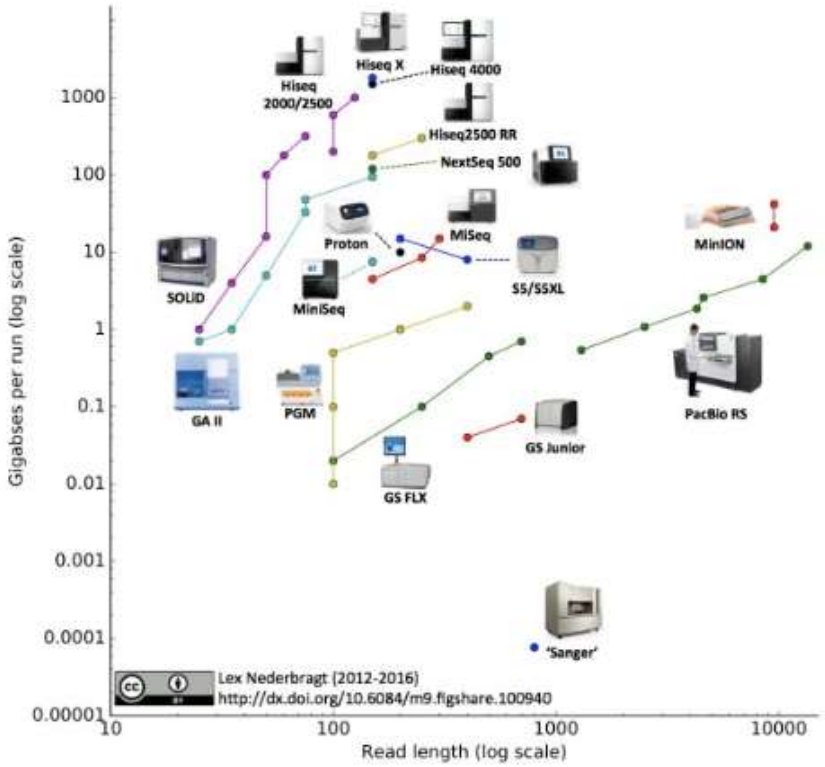
PHOTO: BETTMANN/CORBIS

efficiency of the new methods lies in massive parallelization of the biochemical and measurement steps. The instruments used by Wheeler *et al.* are marketed by 454 Life Sciences, a component of Roche Diagnostics, which joined forces with the Human Genome Sequencing Center at Baylor College of Medicine in Houston, Texas, to sequence Watson's genome.

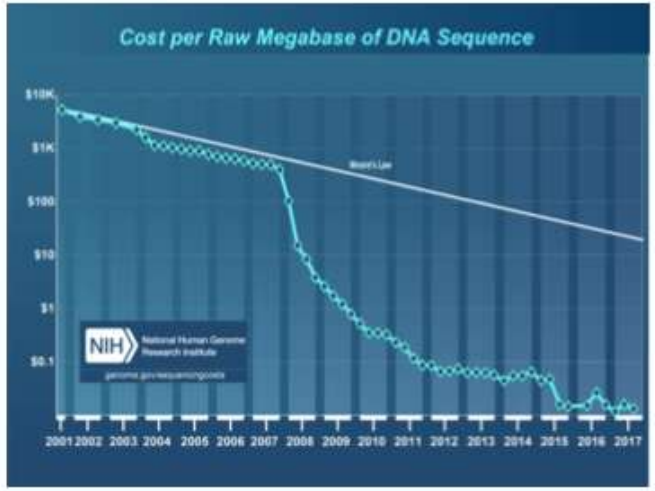
The 454 instruments achieve massive parallelization in two different ways[3]. In an initial step, single DNA molecules are attached to synthetic beads and then amplified enzymatically. During amplification, the beads are trapped in tiny water droplets within a water–oil emulsion; hence, more than 100,000 samples can be processed in parallel in a single test tube. In a later step, during which optical measurements are used to collect the actual sequencing data, each bead is confined to a picolitre-scale well etched into the end of a glass fibre within a fibre-optic bundle. Although costs have not yet dropped to the much-ballyhooed target of US$1,000 per genome[4], they are now low enough to make the era of personal genomics a reality rather than a distant dream.

What can we expect to learn from the sequences of individual genomes? The main lesson from the analyses by Wheeler *et al.* is that it will be extremely difficult to extract medically, or even

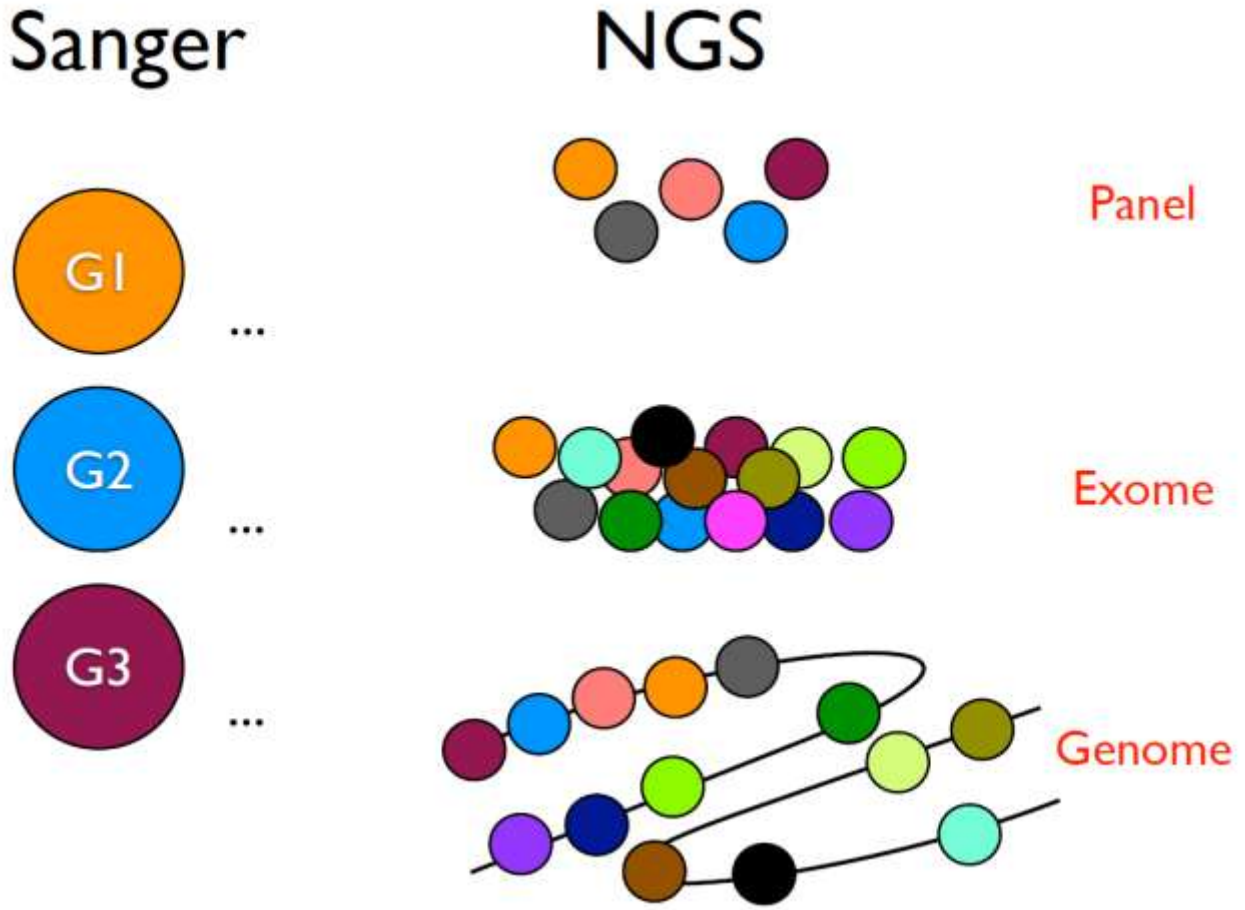
The explosion of DNA sequencing capacity



10M-fold increase in the sequencing capacity (Gigabases per run) from Sanger to today DNA sequencers



Le prospettive cliniche del Progetto Genoma Umano

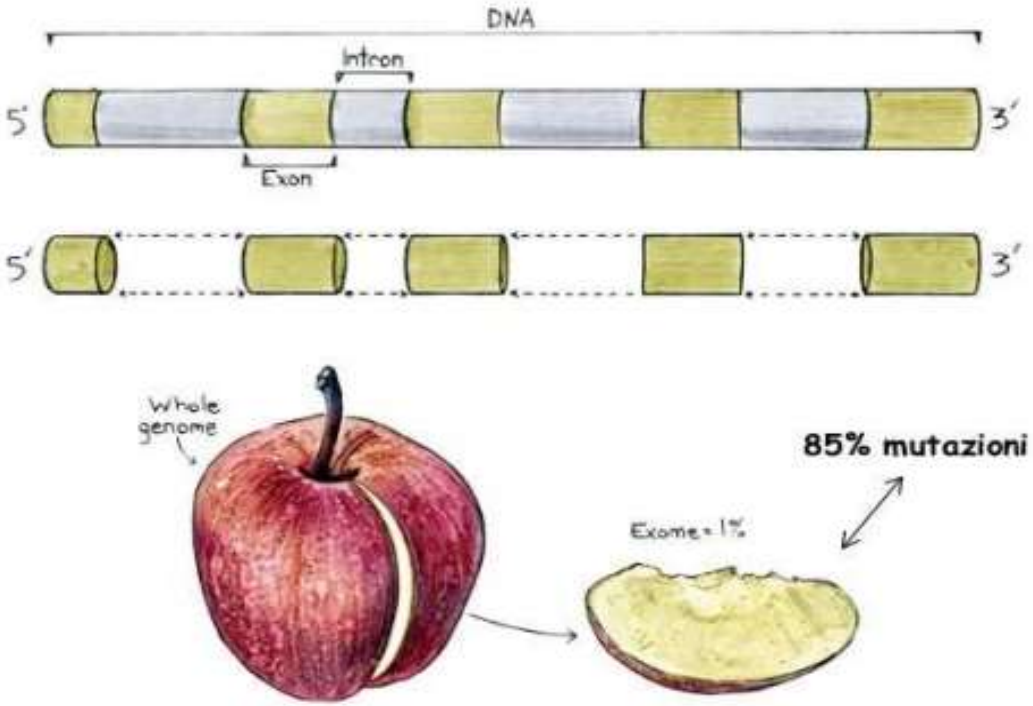


- Malattie Rare
- Frequenza < 1:2.000
- Più di 7.000 descritte
- Nel loro complesso colpiscono il 6-8% della popolazione
- In Italia ne sono affette 2 milioni di persone, con 19.000 nuovi casi l'anno
- La maggior parte ha origine genetica

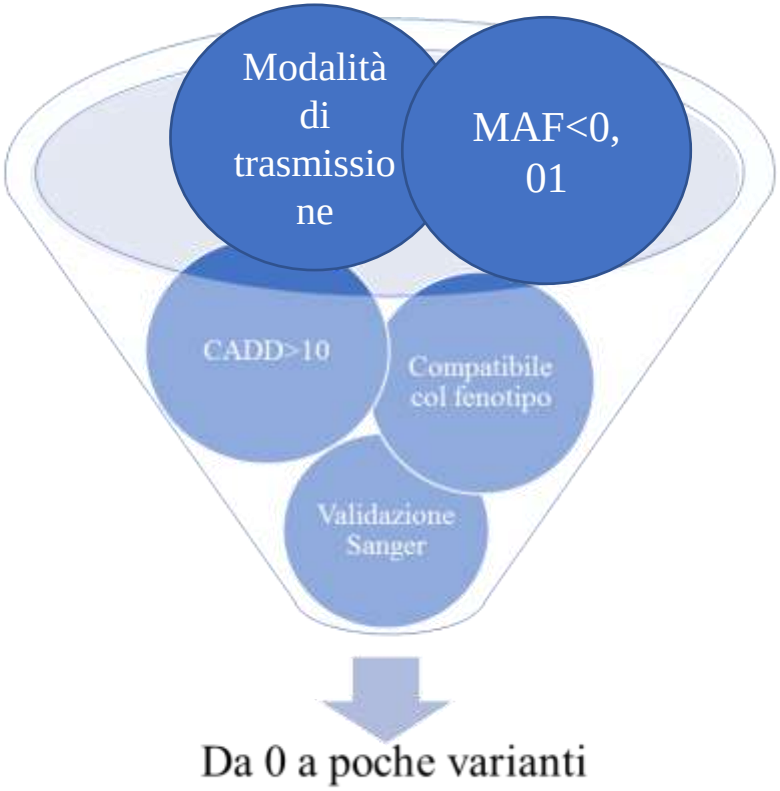
- ULTRARARE < 1:2.000.000
- Potrebbero esserne affette pochissime o una persona al mondo
- Conoscenze estremamente limitate
- Necessità di condivisione dei dati per la loro descrizione e definizione molecolare

Whole Exome Sequencing (WES)

Tecnica NGS in grado di analizzare virtualmente tutte le porzioni codificanti (esoni) di un



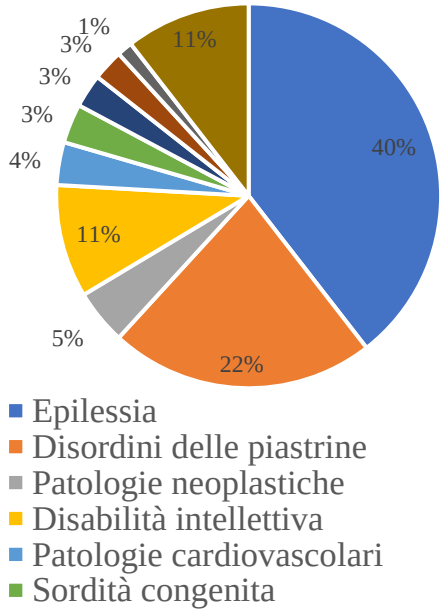
20.000-25.000 varianti



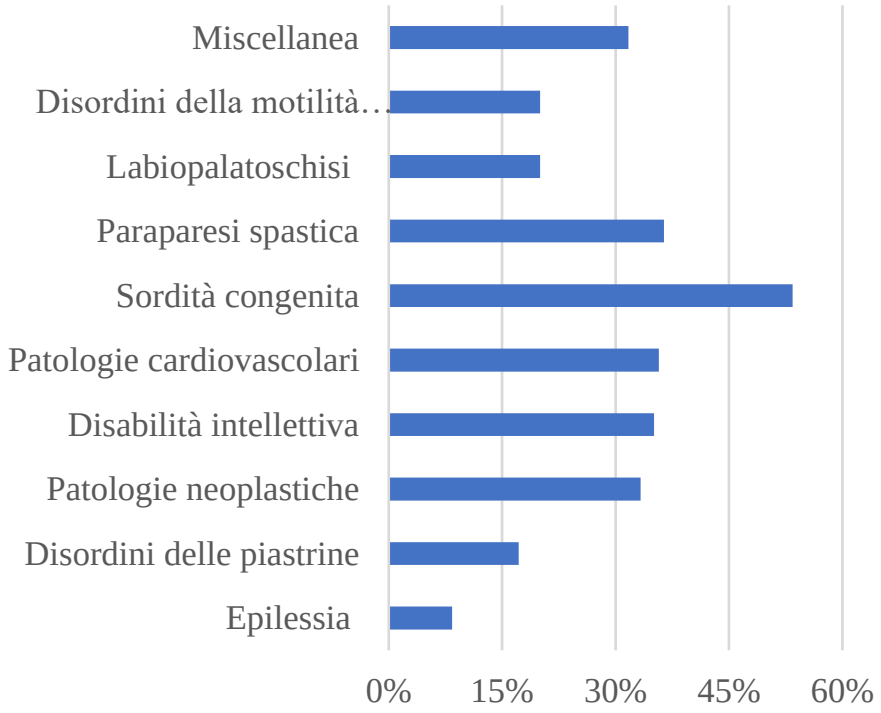
Da 0 a poche varianti

- Resa complessiva del 21,1%
- In caso di analisi di più familiari 29,9%
- Se si escludono le coorti più numerose reclutate per progetti di ricerca 35,4%

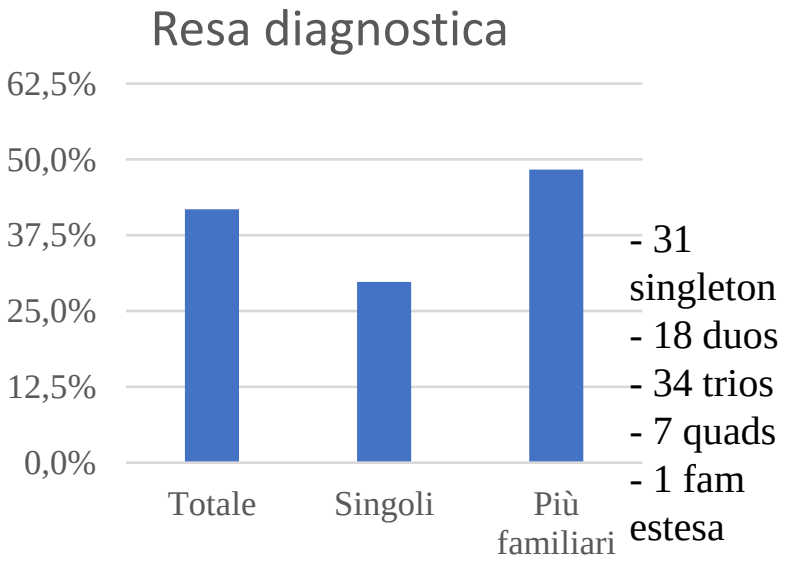
Patologie analizzate

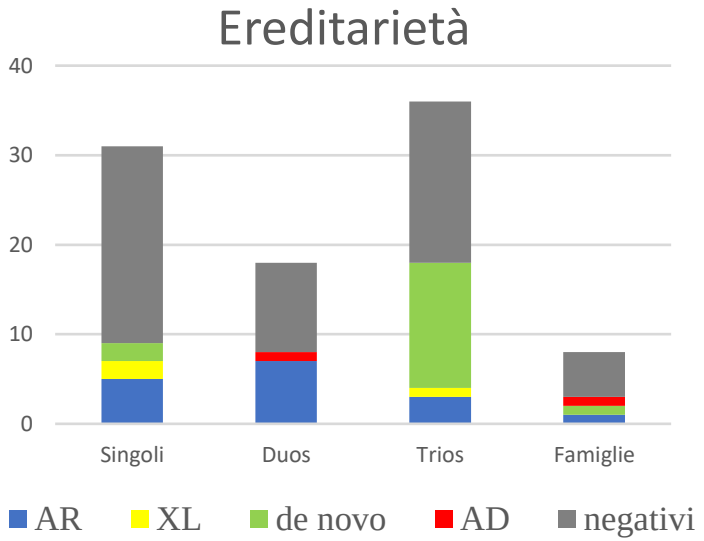


Resa diagnostica per patologia



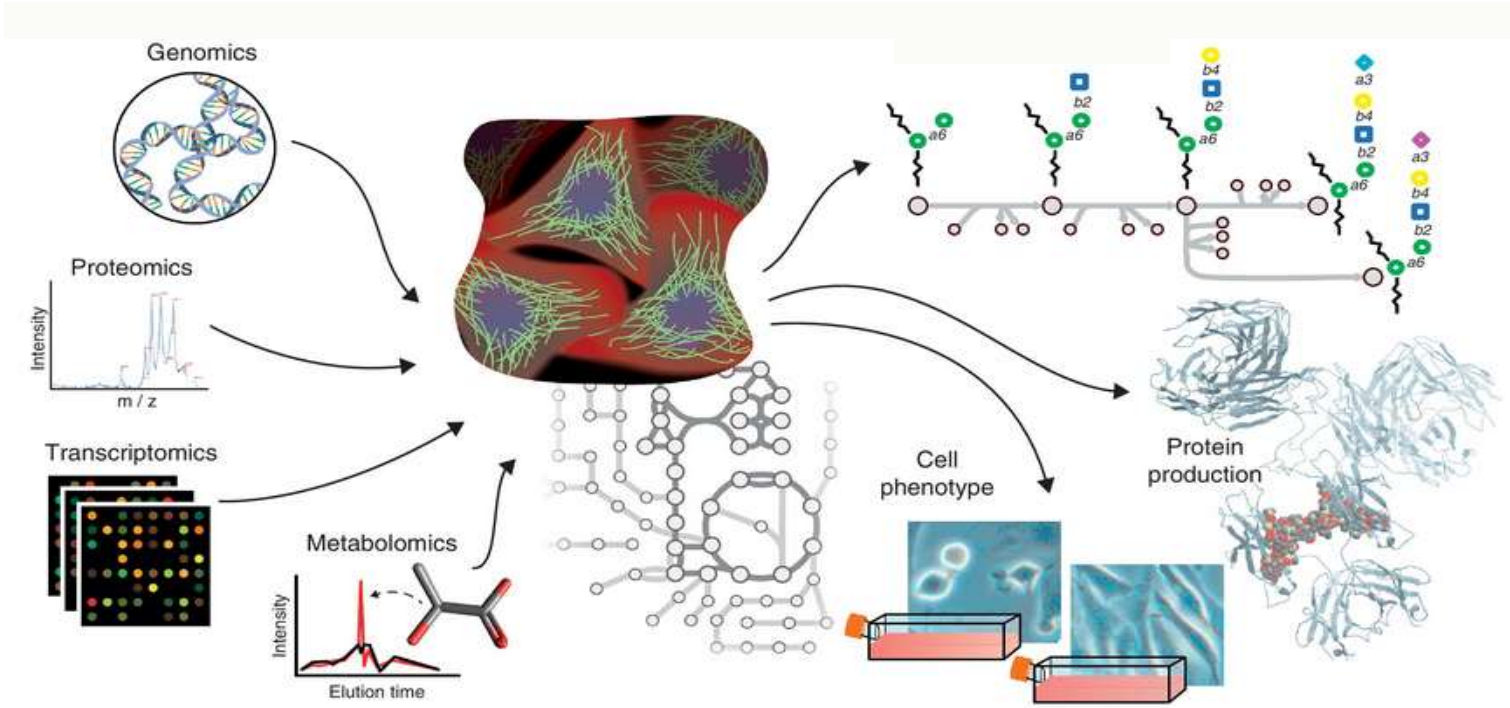
- 91 probandi per un totale di 206 individui
- Tutti avevano eseguito almeno un'altra indagine molecolare 'di primo livello' (prevalentemente CGH-array, sequenziamento Sanger, pannelli NGS…)
- Resa diagnostica 41,8% (38/91 casi), in caso di analisi di più familiari la resa raggiunge il 48,3% (29/60 famiglie)





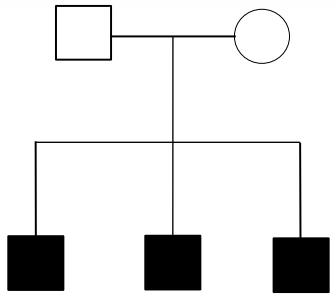
Novel disease-gene correlations

Gene	Disease	Journal	Year
ANKRD26	Thrombocytopenia 2	American Journal of Human Genetics	2011
CACNA2D2	Cerebellar atrophy with seizures and variable developmental delay	PLOS One	2013
PAK3	X-linked syndromic developmental disorder	Human Molecular Genetics	2014
RAD21	chronic intestinal pseudo-obstruction	Gastroenterology	2015
NOTCH3	recessive early-onset arteriopathy and cavitating leukoencephalopathy	EMBO Mol Med	2015
SOS2	Noonan Syndrome	Human Mutation	2015
PRIMA1	nocturnal frontal lobe epilepsy	Annals of Clinical and Translational Neurology	2016
ALDH18A1	Spastic paraplegia 9A, autosomal dominant	Brain	2016
ATAD3A	Harel-Yoon syndrome	American Journal of Human Genetics	2016
ANKRD26	acute myeloid leukemia	Journal of Haematology and Oncology	2017
KIAA1109	Alkuraya-Kucinskas syndrome	American Journal of Human Genetics	2018
TRAPPC2L	Encephalopathy, progressive, early-onset, with episodic rhabdomyolysis	Journal of Medical Genetics	2018
CDC42	Takenouchi-Kosaki syndrome	American Journal of Human Genetics	2018
MYOF	thyroid cancer	Cancer Genetics and Epigenetics	2018
SOX4	Intellectual developmental disorder with speech delay and dysmorphic facies	American Journal of Human Genetics	2019
SMPD4	Neurodevelopmental disorder with microcephaly, arthrogryposis, and structural brain anomalies	American Journal of Human Genetics	2019
NKAP	Intellectual developmental disorder, X-linked syndromic, Hackman-Di Donato type	American Journal of Human Genetics	2019
STARD7	Epilepsy, familial adult myoclonic, 2	Nature Communications	2019
MARCH6	Epilepsy, familial adult myoclonic, 3	Nature Communications	2019
SSBP1	Optic atrophy 13 with retinal and foveal abnormalities	Journal of Clinical Investigations	2020
CCDC32	Cardiofacioneurodevelopmental syndrome	Human Molecular Genetics	2020
SLC12A2	Delpire-McNeill Syndrome	Brain	2020
MAPK1	Noonan syndrome 13	American Journal of Human Genetics	2020
AP1G1	Usmani-Riazuddin syndrome autosomal dominant and recessive	American Journal of Human Genetics	2021
LIG3	Mitochondrial DNA depletion syndrome 20 (MNGIE type)	Brain	2021
SPRED2	Noonan syndrome 14	American Journal of Human Genetics	2021
PIK3C2B	focal epilepsy	Brain	2022
ZMYND8	autosomal dominant neurodevelopmental disorder with cardiac malformations	Genetics in Medicine	2022
NOTCH1	CNS Immune Activation and Microangiopathy	Annals of Neurology	2022
SRSF1	Neurodevelopmental disorder with dysmorphic facies and behavioral abnormalities	American Journal of Human Genetics	2023
CELSR3			



ABBIAMO BISOGNO DI STUDI FUNZIONALI IN MODELLI CELLULARI O IN MODELLI ANIMALI

LIG3



CIPO, problemi autonomici, leucoencefalopatia.

TYMP, ACTG2 neg.

WES: due varianti in eterozigosi composta in tutti i fratelli

PROVE DI PATOGENICITA'

- *LIG3 codifica per la ligasi III, attiva sia nel nucleo sia nel mitocondrio, dove rappresenta l'unica ligasi coinvolta nel riparo del mtDNA, in associazione con la polimerasi γ.*
- Zebrafish *knockout* mostrano alterazione dell'encefalo e della peristalsi intestinale.
- Il fenotipo viene corretto dall'inserimento di *LIG3* wt umano.
- La transfezione con *LIG3* contenente le nostre mutazioni non era in grado di ricostituire il fenotipo normale: mutazioni LOF.
- Studi *in vitro* hanno dimostrato anomalie della catena respiratoria e un aumento delle specie reattive dell'ossigeno.

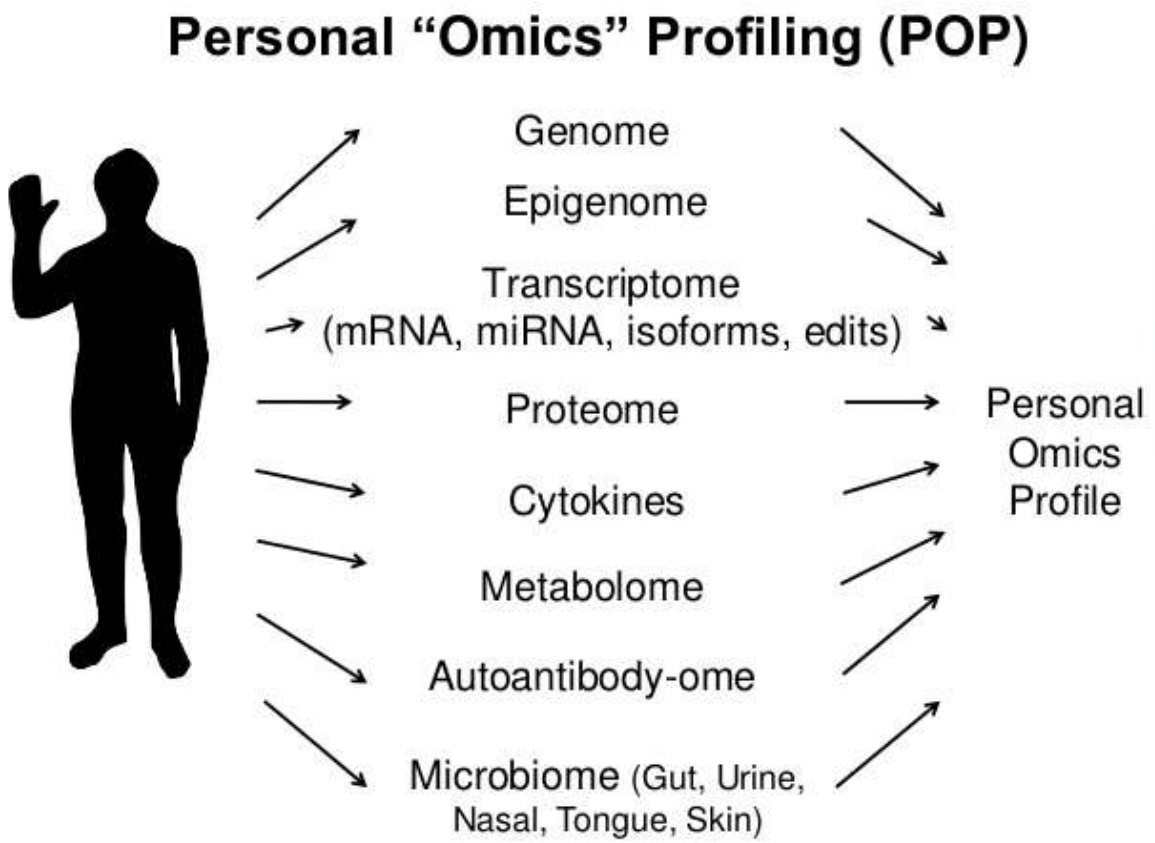
→ Utilizzo di Glutammina per la sua azione antiossidante

- La rivoluzione dell'era Genomica

- L'effetto del progetto genoma umano a livello clinico: l'approccio allo studio delle malattie genetiche rare tramite esoma

- L'utilizzo del genoma nei casi ancora non diagnosticati dopo esoma

- Pazienti negativi all'analisi dell'esoma>>>> **analisi del genoma** (costi/analisi dei dati ancora complessa)



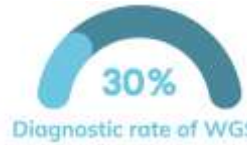
GS increasingly suggested as first-tier clinical test to replace ES for diagnosis of genetic disorders

- In recent years, studies and meta-analyses were published on the topic

European Journal of Human Genetics

The performance of genome sequencing as a first-tier test for neurodevelopmental disorders

Bart P. G. H. van der Sanden, Gaby Schobers, Jordi Corominas Galbany, David A. Koolen, Margje Sinnema, Jeroen van Reeuwijk, Connie T. R. M. Stumpel, Tjitske Kleefstra, Bert B. A. de Vries, Martina Ruiterkamp-Versteeg, Nico Leisten, Michael Kwint, Ronny Derks, Hilde Swinkels, Amber den Ouden, Rolph Pfundt, Tuula Rinne, Nicole de Leeuw, Alexander P. Stegmann, Servi J. Stevens, Arthur van den Wijngaard, Han G. Brunner, Helger G. Yntema, Christian Gilissen, ... Lisenka E. L. M. Vissers



CellPress

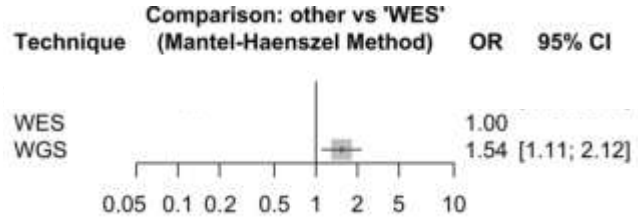
Genome sequencing and comprehensive rare-variant analysis of 465 families with neurodevelopmental disorders

Alba Sanchis-Juan, Karyn Megy, Jonathan Stephens, Camila Armirola Ricaurte, Eleanor Dewhurst, Kayyi Low, Courtney E. French, Detelina Grozeva, Kathleen Stirrups, Marie Erwood, Amy McTague, Christopher J. Penkett, Olga Shamardina, Salih Tuna, Louise C. Daugherty, Nicholas Gleadall, Sofia T. Duarte, Antonio Hedrera-Fernández, Julie Vogt, Gautam Ambegaonkar, Manali Chitre, Dragana Josifova, Manju A. Kurian, Alasdair Parker, Julia Rankin, Evan Reid, Emma Wakeling, Evangeline Wassmer, C. Geoffrey Woods, NIHR BioResource, F. Lucy Raymond, and Keren J. Carss

Archives of Public Health

Whole genome sequencing diagnostic yield for paediatric patients with suspected genetic disorders: systematic review, meta-analysis, and GRADE assessment

Mario Cesare Nurchis, Gerardo Altamura, Maria Teresa Riccardi, Francesca Clementina Radio, Giovanni Chillemi, Enrico Silvio Bertini, Jacopo Garlasco, Marco Tartaglia, Bruno Dallapiccola & Gianfranco Damiani



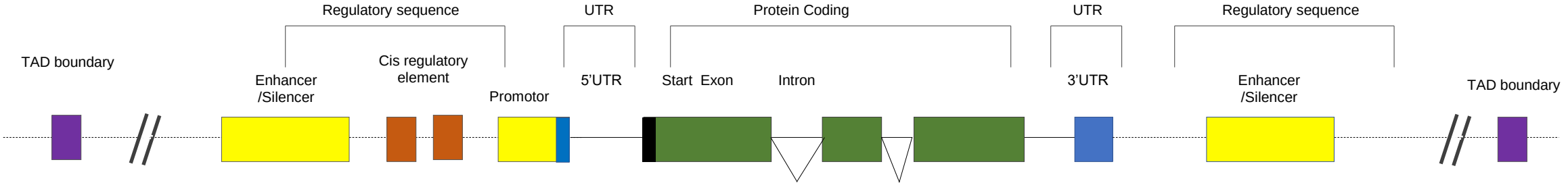
- uniform coverage (including coding)
- comprehensive variant calling
- coding + non-coding

- higher computational and human workload
- more interpretation challenges
- more demanding infrastructure

Genome Sequencing in the clinic

A large portion of non-coding DNA is functional and regulatory elements tightly control gene and protein expression.

- Key elements in the non-coding genome such as promoters, silencer and enhancers ensure that genes are turned on or off at the right moment
- Cis-regulatory elements regulate gene transcription through the binding of transcription factors (TFs)



Mechanisms of which disrupt non-coding elements cause severe disease:

- Splicing, transcription and translation alteration
- RNA processing and stability
- chromatin interactions

Move toward non-coding DNA

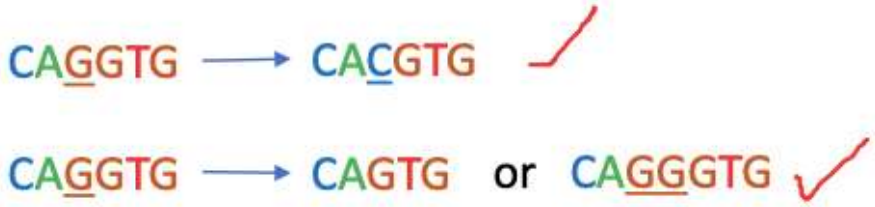
However, interpretation of non-coding regions remains of uncertain significance and more efforts are required to enable a consistent and precise interpretation.

WGS can uncover all type of genetic variation in coding and non-coding DNA in unbiased way.

Well-established

Single Nucleotide Variants

Small Insertion or deletion



Partially studied

Short tandem repeats (STRs)/Repeat expansions (RE)

Copy number Variants (Structural Variant unbalanced)

Balanced structural variants (SVs) – e.g. inversions

Challenges with WGS data

Millions of variants are identified in a typical genome :

- ✓ ~ 4,000,000 of small variants per samples
- ✓ ~ 20,000 Structural Variants for sample
- ✓ ~ 300,000 Short tandem repeats for sample

Challenges with WGS data

Millions of variants are identified in a typical genome :

Questions

1. How do we handle the huge amount of data?

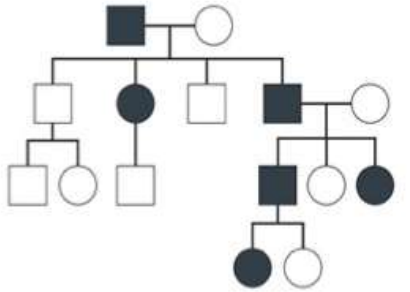
2. How do we facilitate data prioritization for clinical evaluation?

Prioritization: useful steps

Population Frequency

Variant Impact

Inheritance



Gene-Disease association

ClinVar

OMIM
Human Genetics Knowledge for the World

human phenotype ontology

Quality information: coverage

Pathogenicity score

CADD

M-CAP Scores

SIFT score

pLI and Missense Z-score

Article

A draft human pangenome reference

https://doi.org/10.1038/s41586-023-05896-x

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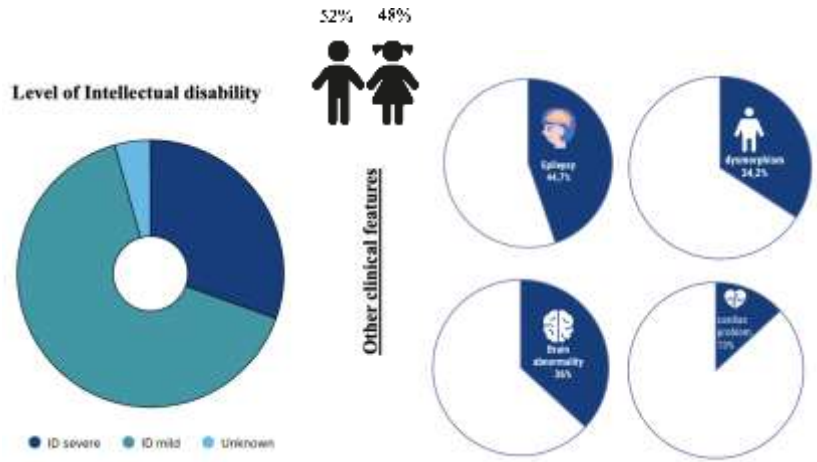
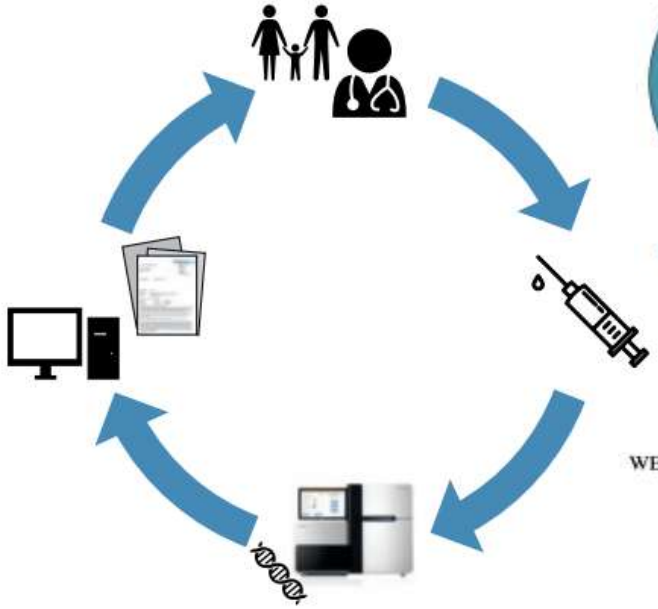
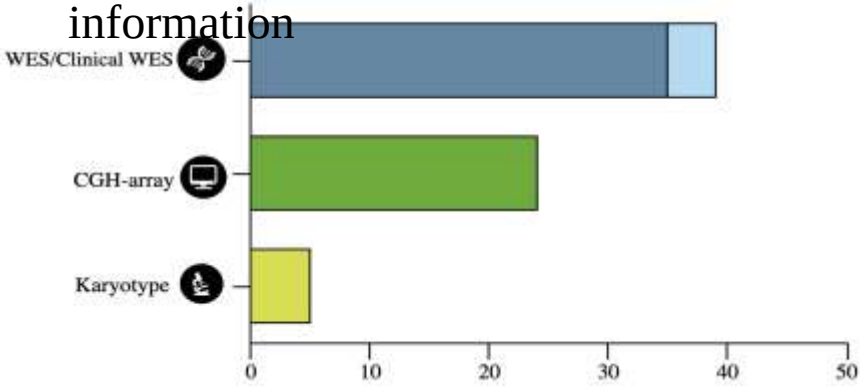
Analysis of 55 families completed
52 trios and 3 quartets



GS data were processed through a DRAGEN/GATK pipeline



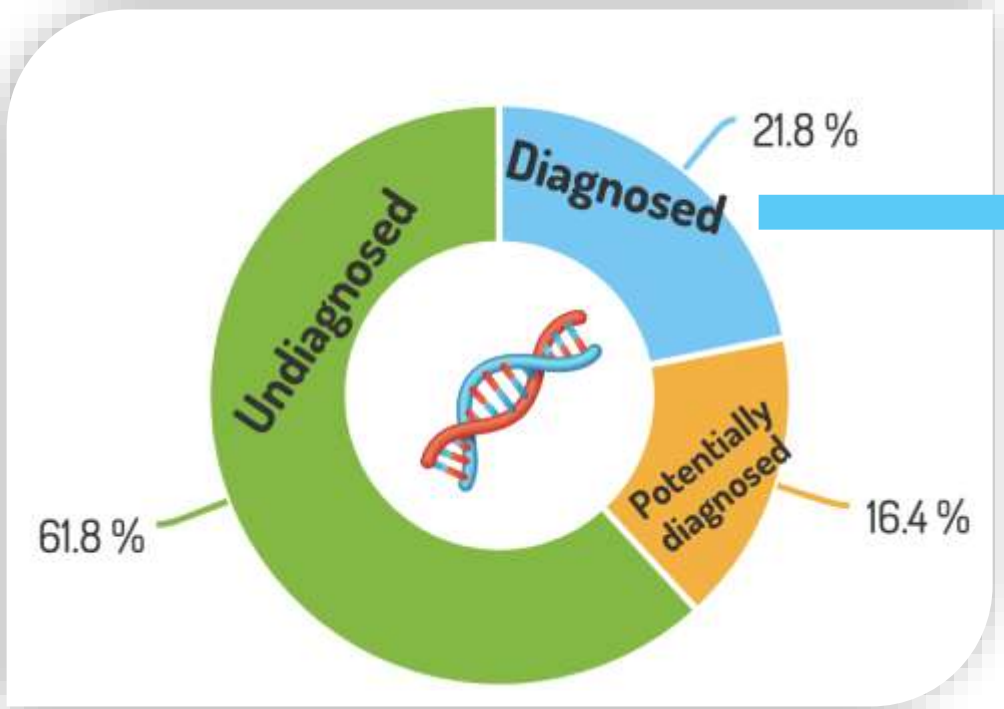
Collect of DNA from blood samples and HPO-standardized clinical information



All family members had GS at mean coverage 50x

Diagnostic rate

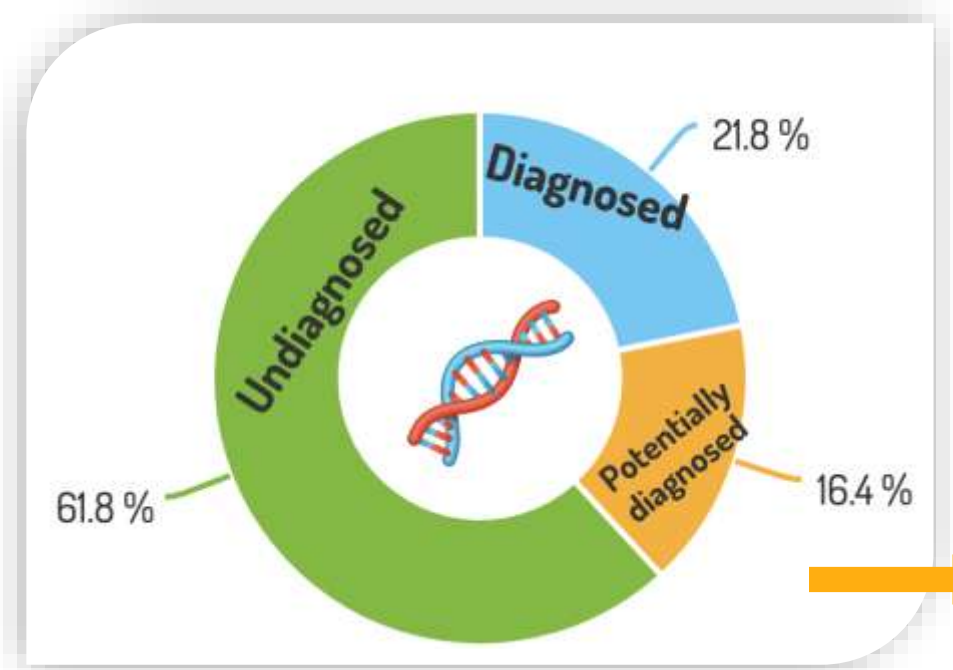
The GS-exclusive variants are highlighted in bold (25%)



The overall diagnostic yield is consistent with studies reported in the literature on cohorts of patients with NDDs (PMID: 36114283, PMID:37541188, PMID37541188)

Case	Gene	Transcript	Variant	Inheritance	type od variant	Potentially detectable with WES
FID_1	CREBBP	NM_004380.3	p.Arg1868Trp	DN	SNV	Y
FID_2	**MT-ATP8**	**ENST00000361851.1**	**p.Lys57***	**MT**	**SNV**	N
FID_3	**RIF1**	**NM_018151.5**	**2-151426560-6821_del**	**DN**	**SV**	**N**
FID_4	**MED12**	**NM_005120.3**	**c.4477_4527+56dup**	**DN**	**SV**	**N**
FID_7	HECW2	NM_001348768.2	p.Arg1330Trp	DN	SNV	Y
FID_8	AFF4	NM_014423.4	p.Arg258Trp	DN	SNV	Y
FID_22	CYFIP2	NM_001037333.3	p.Asp877GlufsTer57	DN	SNV	Y
FID_10	TRIT1	NR_132405.1	p.Trp228Arg, p.Arg150Ter	AR	SNV	Y
FID_38	SCAMP5	NM_138967.4	p.Gly180Trp	AD	SNV	Y
FID_31	RAC1	NM_006908.5	p.Tyr64His	DN	SNV	Y
FID_45	ZNF865	NM_001195605.2	p.(Ser800PhefsTer163)	DN	SNV	Y
FID_47	DDX1	NM_004939.3	p.(Glu371Lys)	DN	SNV	Y

Diagnostic rate



Case	Gene	Transcript	Variant	Inheritance	type od variant	genomic region	Potentially detectable with WES
FID_29	HDLBP	NM_005336.5	p.Arg839His	DN	SNV new gene	Coding	Y
FID_18	CHRDL1	NM_001143981.2	p.Lys300Ter	XLR	SNV	coding	Y
	FGF13	**NM_004114.5**	**c.-25A>C**	**XLR**		**non-coding**	**N**
FID_19	**MT-RNR2**	**ENST00000387347**	**M-3251-A-G**	**MT**	**SNV**		**N**
	NLGN4X	**ENST00000381095.8**	**X:5863700+300_ins**	**XLR**	**SV**	**non-coding**	**N**
FID_11			**inv(X)(p22.13q28)**	**DN**	**SV**	**non-coding**	**N**
	GCH1	NM_001024024.1	p.Thr94Met	DN	SNV	Coding	Y
FID_39	**FGF14/TLX1NB**		**t(10,13);**	**DN**	**SV**	**Coding**	**N**
	SYNJ1	**ENST00000674351.1**	**p.Pro1255Leu c.*2436A>G**	**AR**	**SNV**	**Coding**	**N**
FID_50	PIK3R2	NM_005027.4	(Glu338Gly)	M	SNV	Coding	Y
	GPC3	**NM_004484.4**	**c.175+4751A>**	**XLR**	**SNV**	**non-coding**	**N**
	OGT	**NM_181672.3**	**c.218+56A>G**	**XLR**	**SNV**	**non-coding**	**N**
FID_54	SRCAP	NM_006662.3	p.(Arg945Leu)	AD	SNV	Coding	Y
FID_42	COL23A1	NM_173465.4	p.Arg495Cys	AR	SNV/ROH gene nuovo ma paziale phen	Coding	Y
FID_25	TENM2	ENST00000518659.6		AD	SNV gene nuovo in un CRE	non-coding	N

AOU

alla nascita: P -0.43 DS, L -0.9 DS, CC -0.7 DS

brevità assoluta del funicolo ombelicale

grave ritardo nelle tappe di sviluppo psicomotorio, severa I.D., deficit di coordinazione dinamica e deficit prassico-motori.

cheilo-gnato-palatoschisi

atresia dell'arteria polmonare e DIV

coloboma irideo bilaterale con coinvolgimento del nervo ottico, OO D

exotropia e lagoftalmo, nistagmo a più componenti,

funzionalità visiva compromessa; ipoacusia trasmissiva;

fossa nasale sinistra substenotica per deviazione del setto nasale;

displasia congenita dell'anca sinistra; scoliosi

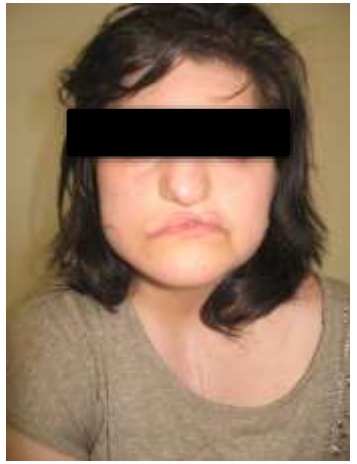
imene imperforato; amenorrea secondaria.

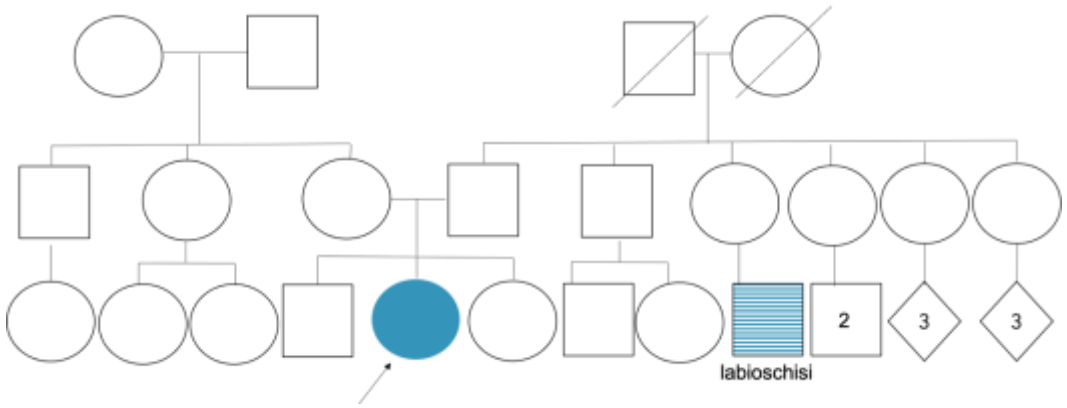
deformità scapolare di Sprengel;

paresi del VII nervo cranico dx

TC encefalo: ipoplasia del verme cerebellare.

D. 29 anni





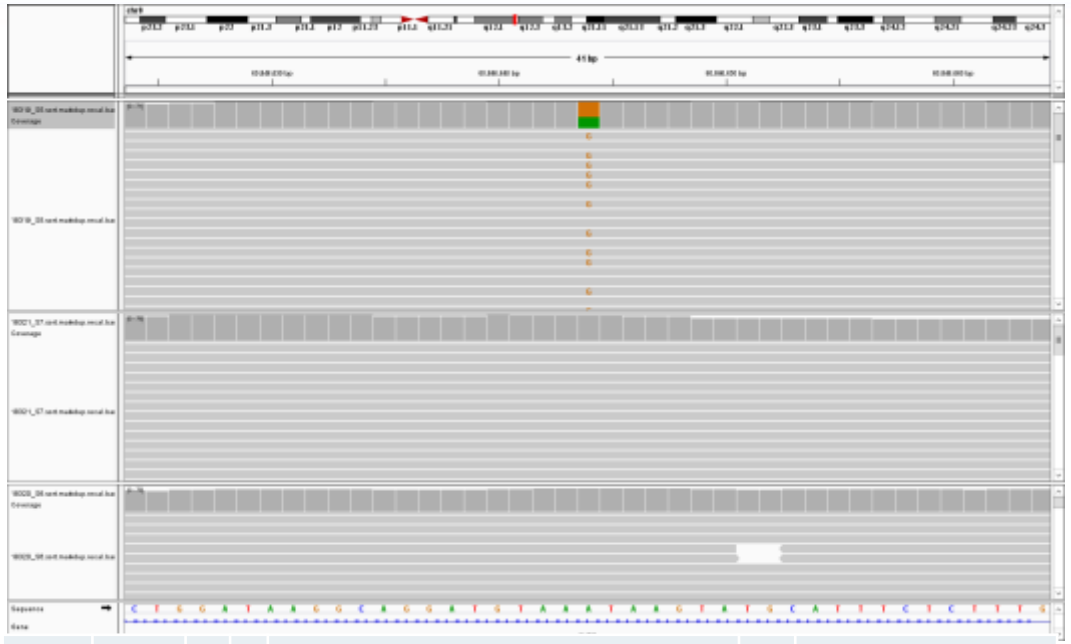
Sospetto clinico di Sindrome di **CHARGE**

Coloboma dell'iride, della retina e/o del disco ottico
difetti cardiaci (**H**eart), **A**tresia delle coane,
Ritardo di crescita e dello sviluppo,
ipoplasia dei **G**enitali,
anomalie dell'orecchio interno ed esterno (e ipoacusia) (**E**ar)

LE INDAGINI GENETICHE CONDOTTE NEL PERCORSO DIAGNOSTICO

- cariotipo su sangue periferico
- array-CGH (risoluzione media 120kb)
- analisi del gene *CHD7*
- Whole Exome Sequencing in modalità trio.

c.5210+1235A>G de novo *CHD7*



chrom	pos	ref	alt	name	strand	change
chr8	61759203	A	G	ENST00000423902_exon_22_0_chr8_61757809_f	+	NM_017780.3:c.5210+1235A>G

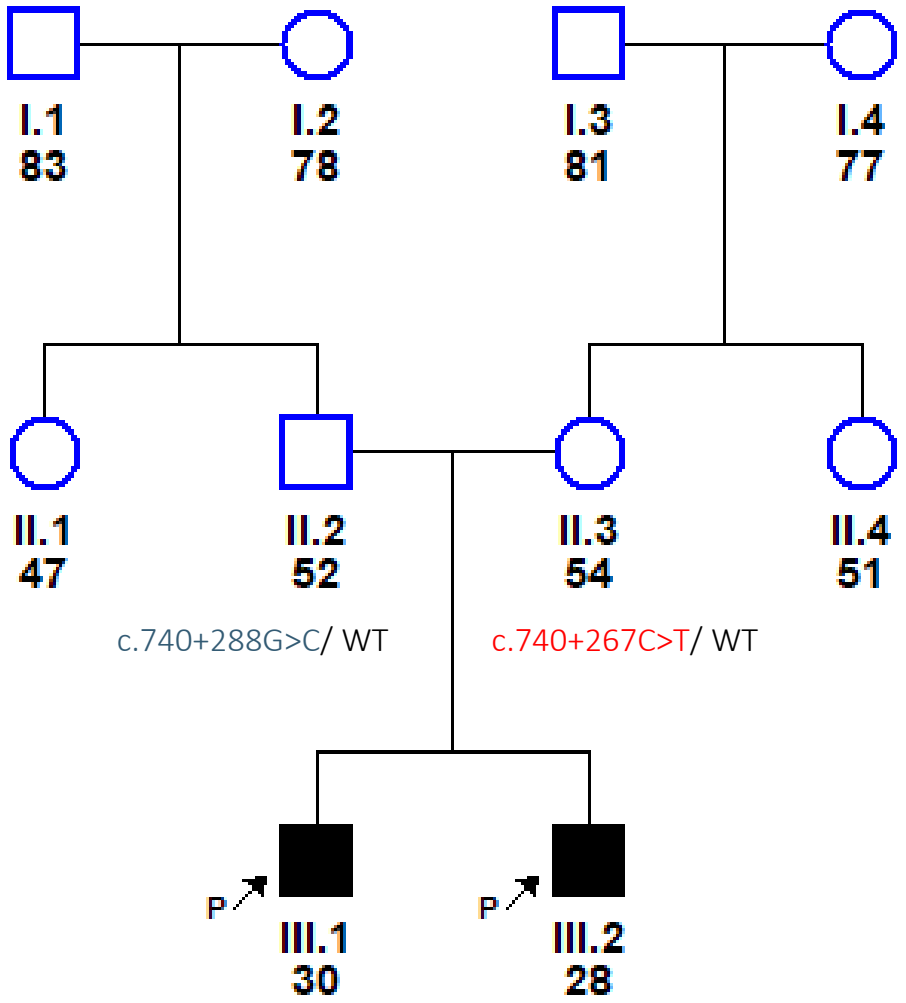
RegSNPs-intron:

DISEASE	PROB	TRUE POSITIVE RATE	FALSE POSITIVE RATE	SPLICING SITE
D	0.75	0.13	0	off

WGS	c.5210+1235A>G de novo CHD7

III.1
- Hemeralopia: onset at 20 yo
- Nicturia and hypertension at 27 yo: ESRD diagnosis
- Kidney biopsy suggestive of Nephronophthisis
- Kidney transplantation at 29 yo

Eye examination (2021):
- Fundus: attenuated retinal vessels, waxy optic nerve pallor, salt and pepper peripheral retinopathy
- OCT: extrafoveal photoreceptors atrophy
- Rod – cone dystrophy

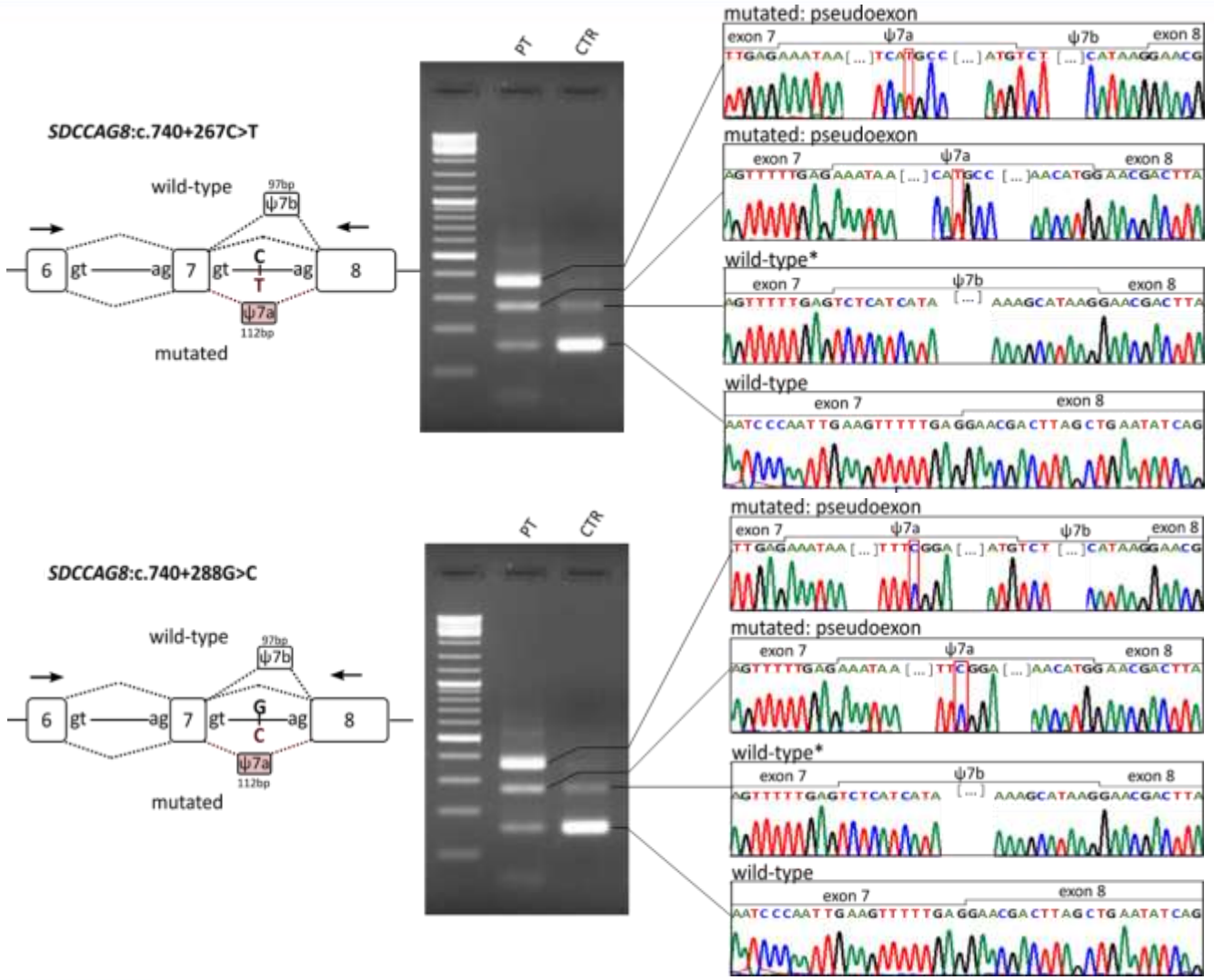


Family history

III.2
- Hemeralopia: onset at 20 yo
- Hypertension
- Mild renal failure

Eye examination (2021):
- Fundus: bone spicule pigment deposits and salt and pepper peripheral retinopathy
- OCT: extrafoveal photoreceptors atrophy
- Rod – cone dystrophy

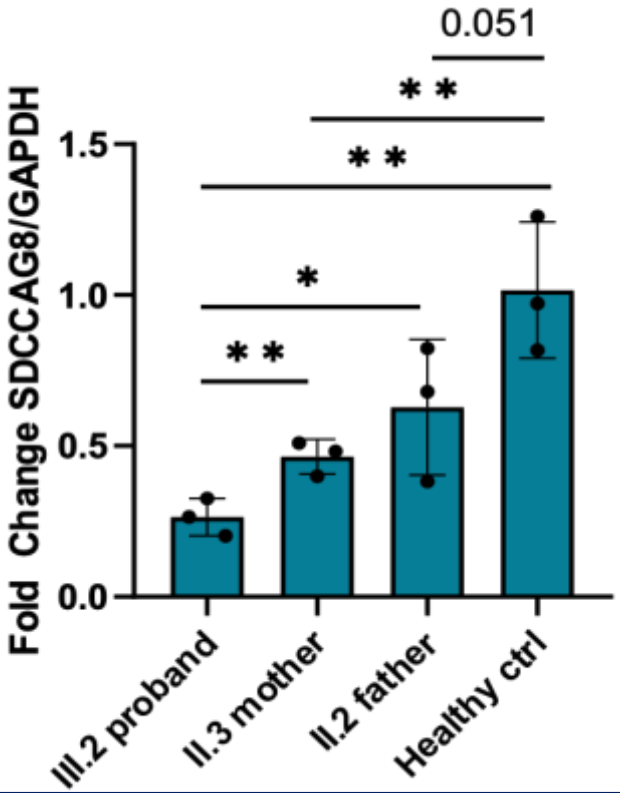
In vitro validation of cryptic variants



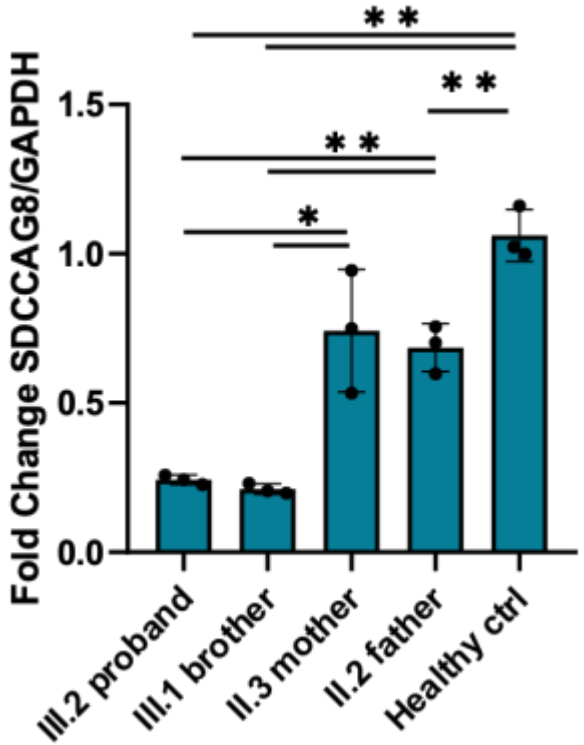
By RT-PCR experiments on cDNA derived from patients' fibroblasts, we characterized the splicing anomalies associated with the c.740+267C>T and the c.740+288G>C deep intronic variants in the *SDCCAG8* gene. Both variants alter an ESE/ESS motif, causing the creation of a 112bp pseudoexon (Ψ7a). Interestingly, two wild-type splicing isoforms have been detected (7-8 and 7- Ψ7b-8).

Testing the effect of our *SDCCAG8* variants on splicing

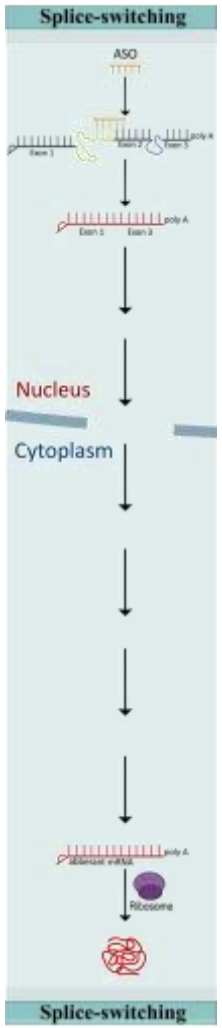
RNA SOURCE: URINARY STEM CELLS



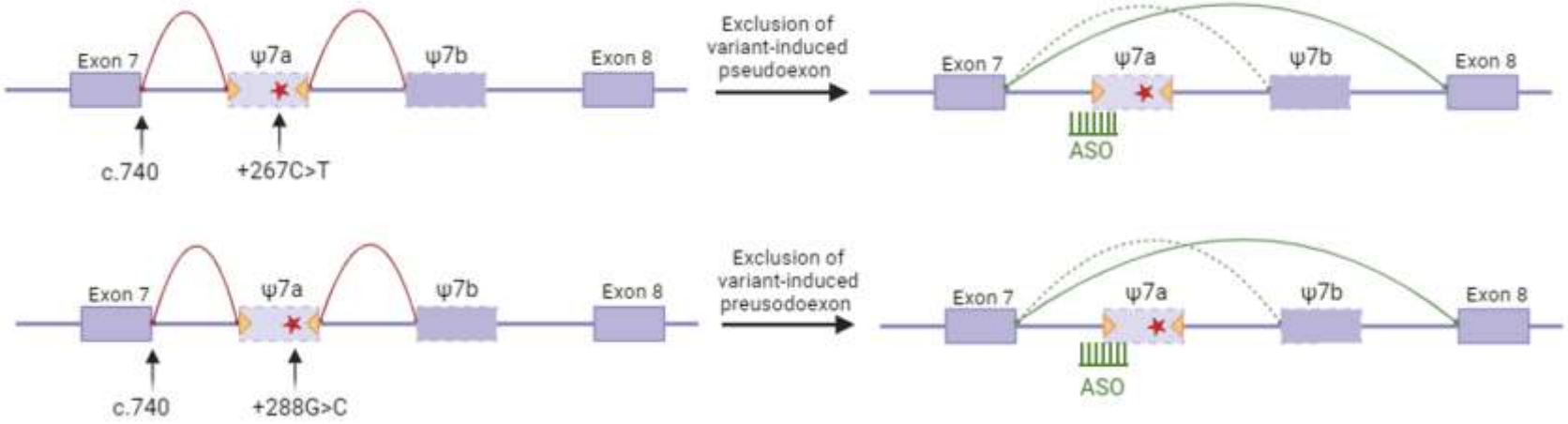
RNA SOURCE: SKIN FIBROBLASTS



qRT-PCR experiments show significant reduction of the WT transcript in affected patients vs their parents and the healthy control on t-test (* $p < 0.05$; ** $p < 0.01$), in skin fibroblast and urinary stem cells

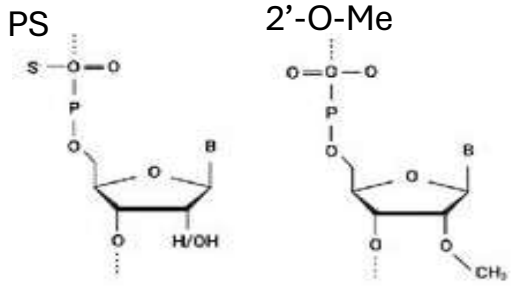


Our ASO strategy to target *SDCCAG8* cryptic variants.



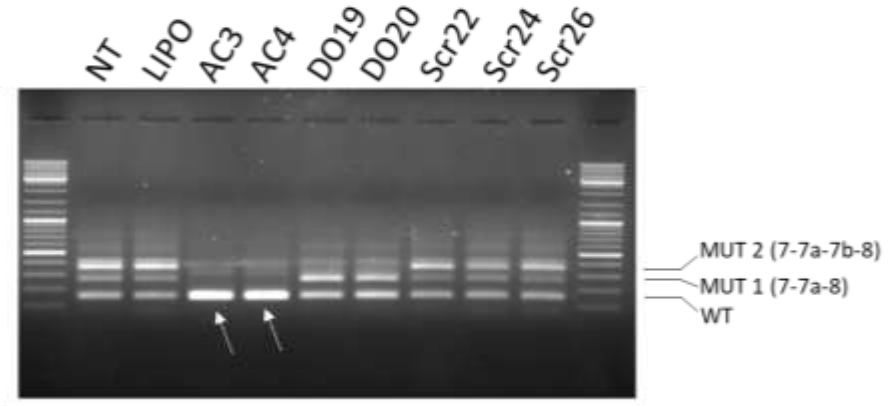
We designed and synthetized four splice-switching ASO to restore normal *SDCCAG8* splicing at the pre-mRNA level.

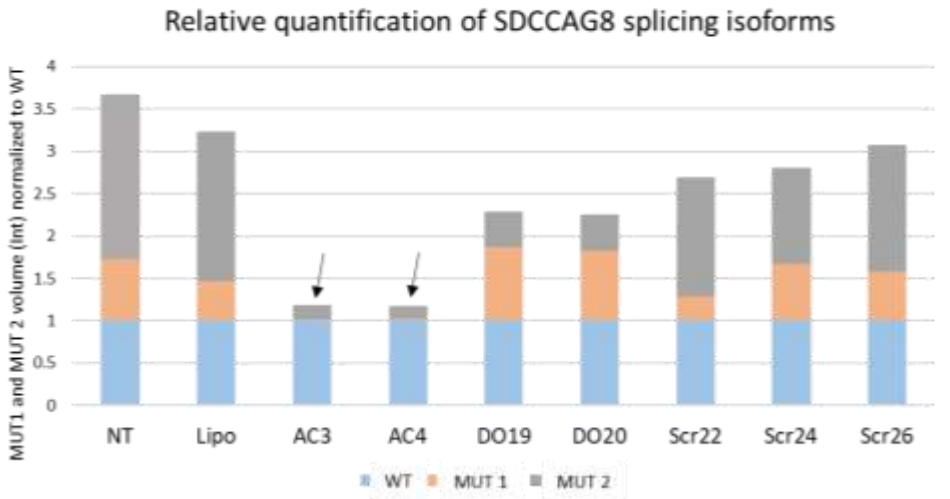
To the molecules we have added a 2'-O-methyl RNA phosphorothioate (2OMePS) **chemical modification** in order to increase stability and improves the affinity for the target sequence.



In vitro evaluation of ASOs therapeutic potential

Patient fibroblasts were treated with 20, 35, 50 and 65 nM concentrations of ASOs for 48h. Total RNA was isolated and RT-PCR was performed to evaluate the therapeutic potential of our molecules.





Our preliminary experiments show the efficacy of AC3 and AC4 ASOs in restoring the physiological splicing pattern (arrows) at all concentration tested. The treatments dramatically improved *SDCCAG8* splicing correction of pre-mRNA.

The relative quantification of MUT 1 and MUT 2 splicing isoforms *vs* the WT one clearly states the therapeutic potential of our molecules, indeed mutant bands had almost disappeared, suggesting a complete abrogation of the aberrant splicing.

Consulenza genetica

Processo di comunicazione

aspettative

storia familiare

dubbi

sentimenti

richieste

bidirezionale

Non-direttivo

"educazione"

probabilità di eredo-familiarità

rischio individuale

opzioni preventive

Grazie per l'attenzione!