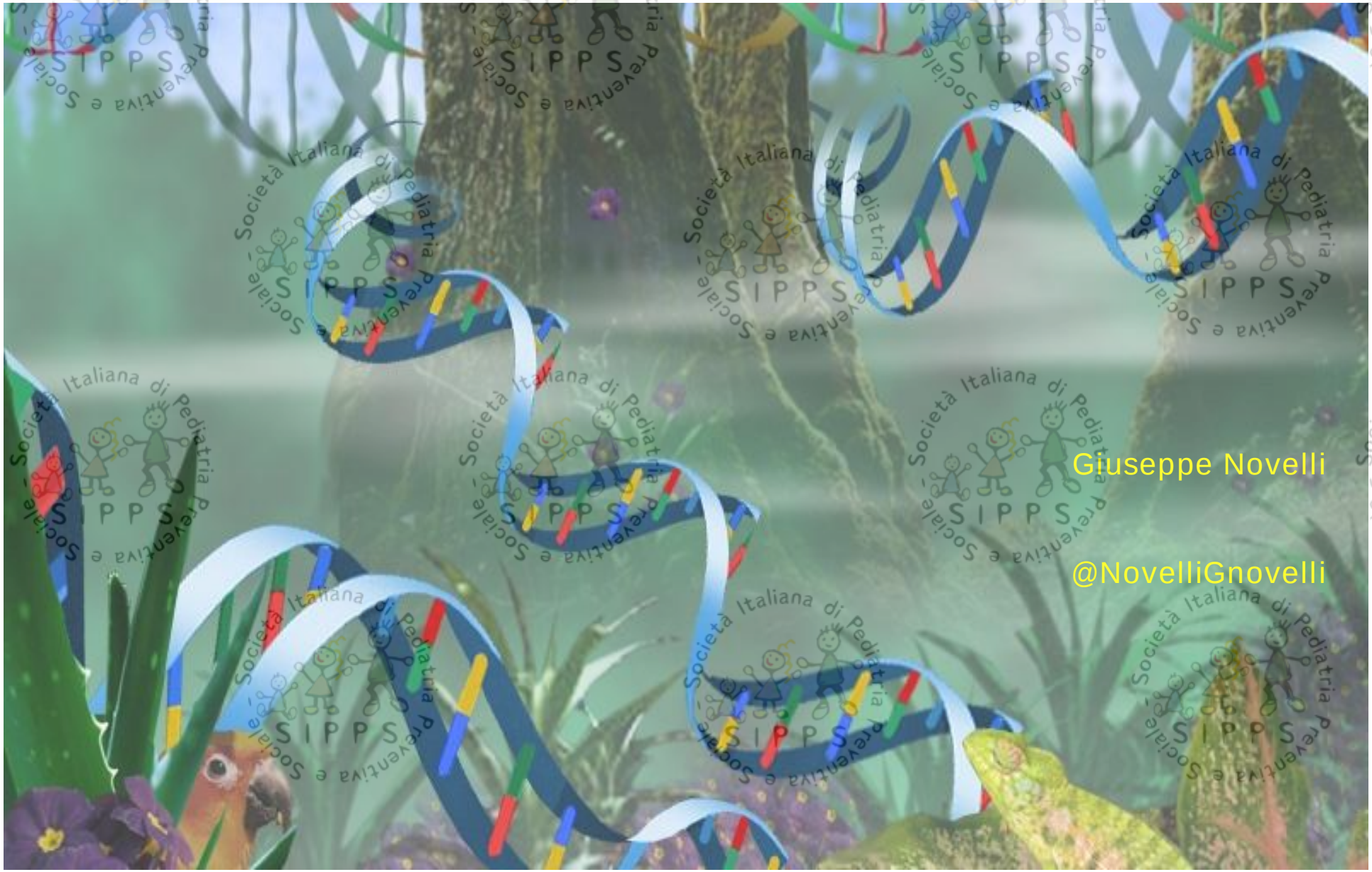
Università di Roma Tor Vergata

Centro di Eccellenza per lo Studio del Rischio Genomico in Patologie Complesse Multifattoriali – Facoltà di Medicina e Chirurgia



Giuseppe Novelli

@NovelliGnovelli

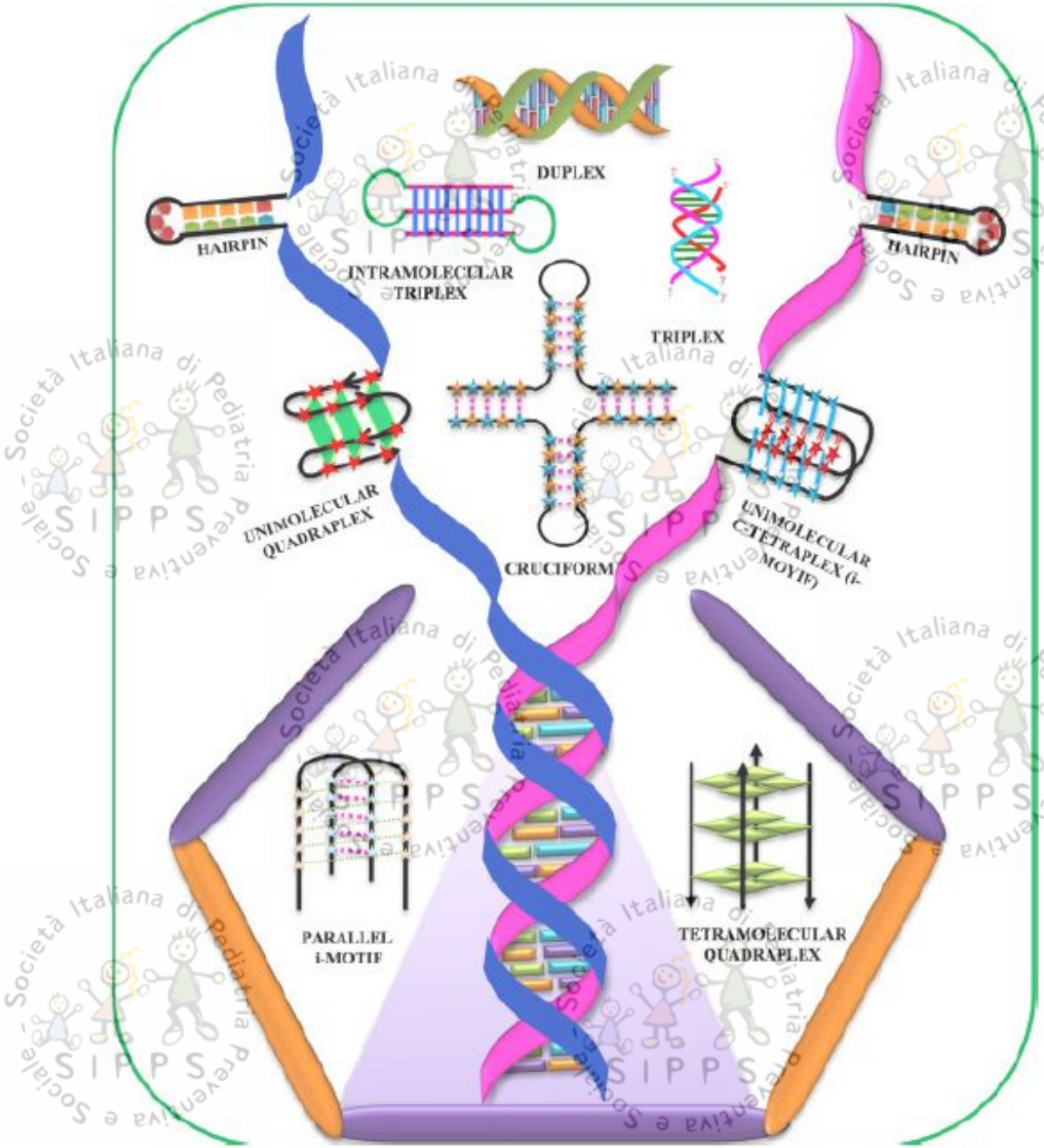


Fig. 1. Bouquet of non-canonical DNA structures.

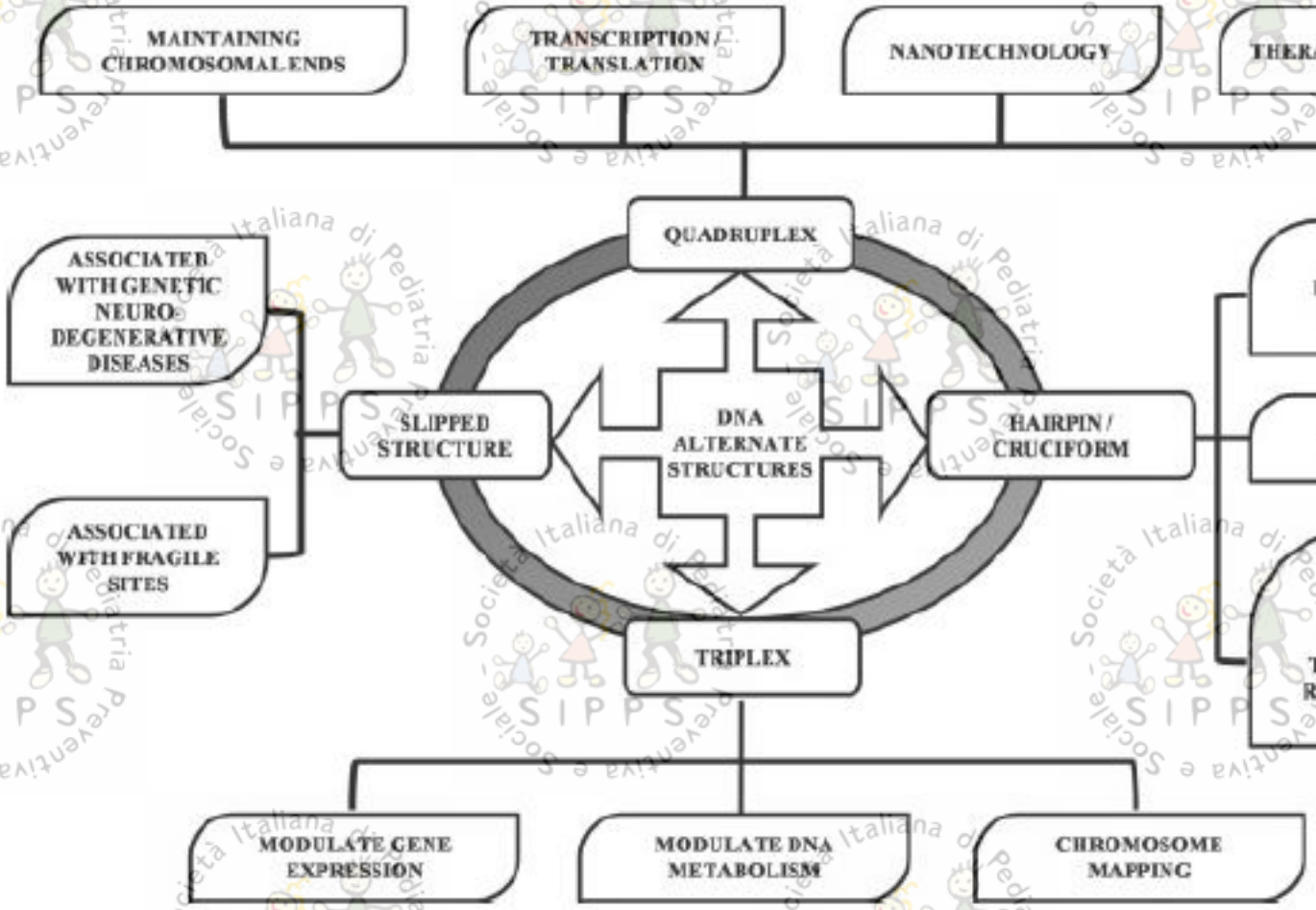


Fig. 2. Pictorial representation of biological applications of non-canonical DNA structures.

"…human genes are not ordinary molecules but are fundamentally unique because they transmit the instructions for creating humans…"

DNA on Chromosome 7

GAAATAATTAATCTTTTCCTTCCTTCTCCTATTTTGTCCTTTACTTCAATTTATTTATTTATTATTAATATTATTATTTTTTG
AGACGGAGTTTC/ACTCTTGTTGCCAACCTGGAGTGCAGTGGCGTGATCTCAGCTCACTGCACACTCCGCTTTCCTG
GTTTCAAGCGATTCTCCTGCCTCAGCCTCCTGAGTAGCTGGGACTACAGTCACACACCACCACGCCCGGCTAATTTTT
GTATTTTTAGTAGAGTTGGGGTTTCACCATGTTGGCCAGACTGGTCTCGAACTCCTGACCTTGTGATCCGCCAGCCTC
TGCCTCCCAAAGAGCTGGGATTACAGGCGTGACCCACCGCGCTCGGCCCTTTGCATCAATTTCTACAGCTTGTTTTCT
TTGCCTGGACTTTACAAGTCTTACCTTGTTCTGCC/TTCAGATATTTGTGTGGTCTCATTCTGGTGTGCCAGTAGCTAA
AAATCCATGATTTGCTCTCATCCCACTCCTGTTGTTCATCTCCTCTTATCTGGGGTCACA/CTATCTCTTCGTGATTGC
ATTCTGATCCCCAGTACTTAGCATGTGCGTAACAACTCTGCCTCTGCTTTCCCAGGCTGTTGATGGGGTGCTGTTCAT
GCCTCAGAAAAATGCATTGTAAGTTAAATTATTAAAGATTTTAAATATAGGAAAAAAGTAAGCAAACATAAGGAACAA
AAAGGAAAGAACATGTATTCTAATCCATTATTTATTATACAATTAAGAAATTTGGAAACTTTAGATTACACTGCTTTTA
GAGATGGAGATGTAGTAAGTCTTTTACTCTTTACAAAATACATGTGTTAGCAATTTTGGGAAGAATAGTAACTCACCC
GAACAGTG/TAATGTGAATATGTCACTTACTAGAGGAAAGAAGGCACTTGAAAAACATCTCTAAACCGTATAAAAAC
AATTACATGATAATGATGAAAACCCAAGGAATTTTTTTAGAAAACATTACCAGGGCTAATAACAAAGTAGAGCCACAT
GTCATTTATCTTCCCTTTGTGTCTGTGTGAGAATTCTAGAGTTATATTTGTACATAGCATGGAAAAATGAGAGGCTAGT
TTATCAACTAGTTCATTTTTAAAAGTCTAACACATCCTAGGTATAGGTGAACTGTCCTCCTGCCAATGTATTGCACATT
TGTGCCCAGATCCAGCATAGGGTATGTTTGCCATTTACAAAGCTTTATGTCTTAAGAGAGGAAATATGAAGAGCAAAA
CAGTGCATGCTGGAGAGAGAAAGCTGATACAAATATAAAT/GAAACAATAATTGGAAAAATTGAGAAACTACTCATT
TTCTAAATTACTCATGTATTTTCCTAGAATTTAAGTCTTTTAATTTTTGATAAATCCCAATGTGAGACAAGATAAGTATT
AGTGATGGTATGAGTAATTAATATCTGTTATATAATATTCATTTTCATAGTGGAAGAAATAAAATAAAGGTTGTGATGA
TTGTTGATTATTTTTTCTAGAGGGGTTGTCAGGGAAAGAAATTGCTTTTT

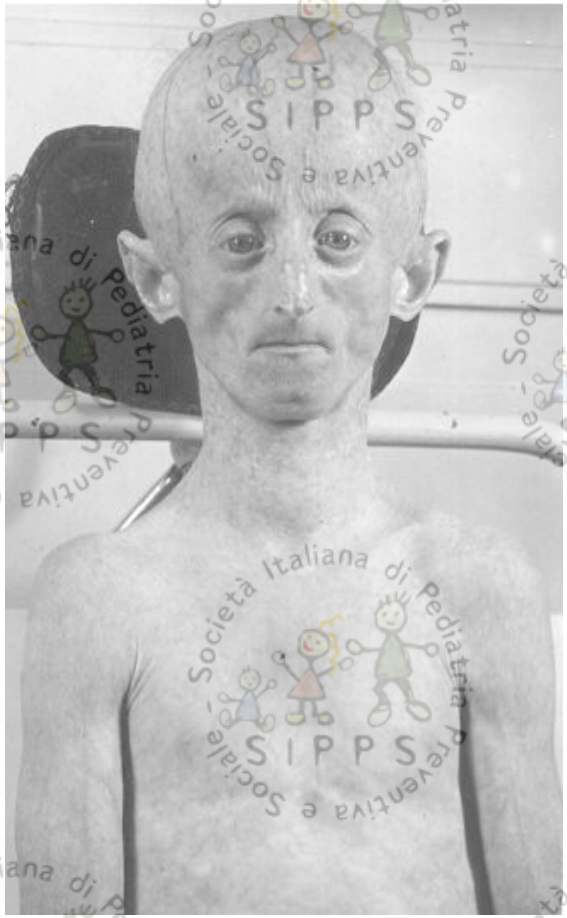
SNPs 1 / 300 bases


DECEMBER 24, 2012
Egypt Divided / Pot's Big Moment / Best of 2012
Movies, Music, Books & More
TIME
Want to Know My Future?
Alzheimer's
Diabetes
Asthma
Colon cancer
Breast cancer
Huntington's disease
Epilepsy
Parkinson's
Glaucoma
Dementia
Cancer
Hemochromatosis
Cystic fibrosis
Tay-Sachs disease
Burkitt's lymphoma
Malignant melanoma
Phenylketonuria
Prostate cancer
Obesity
New genetic tests can point to risks—but not always a cure
BY BONNIE ROCHMAN
www.time.com


AP

PROGERIA - HGPS

10 months



14 yr

Hutchinson, Medicochirurgical Trans 1886;69:473

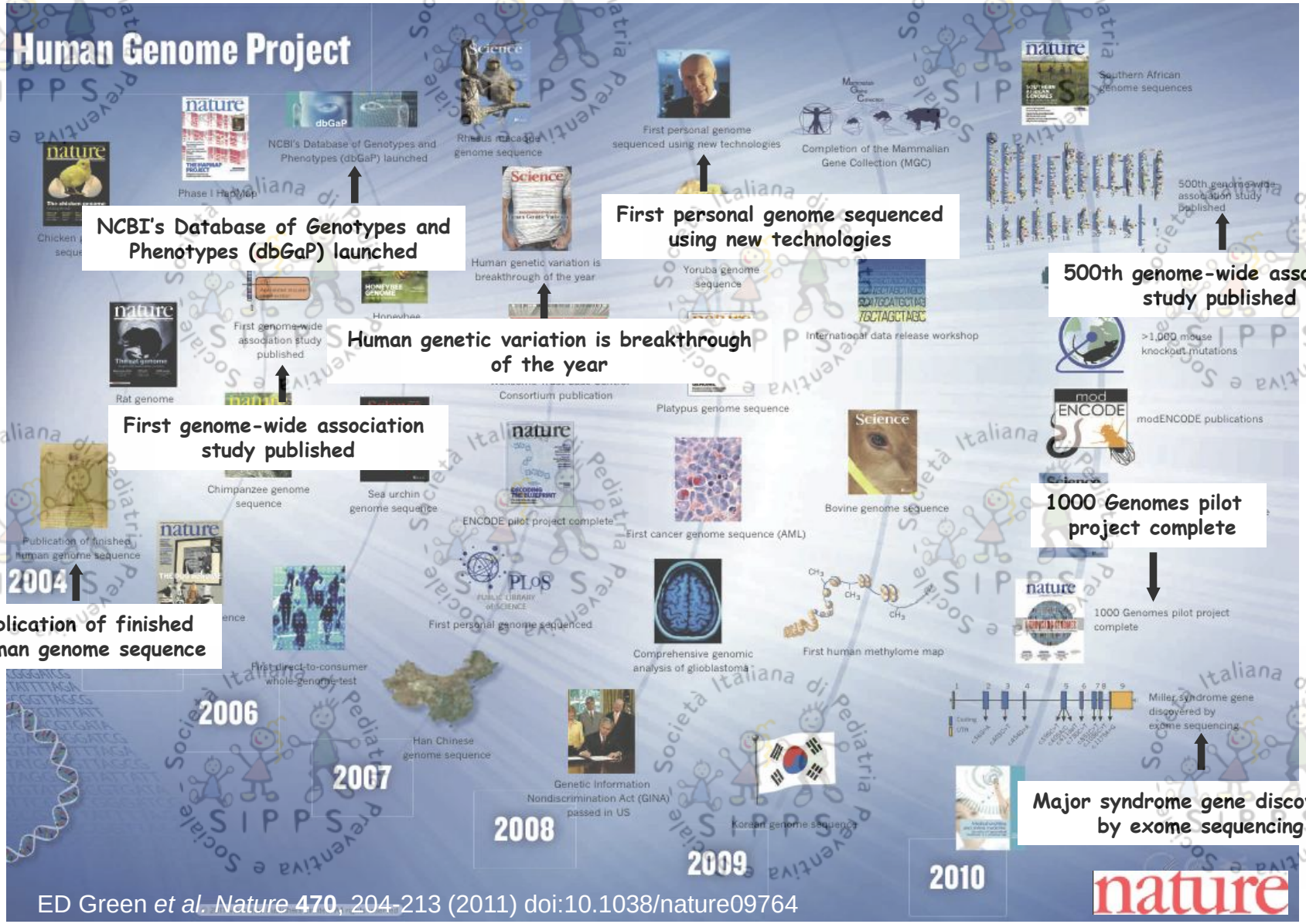
Gilford, Practitioner 1904;73:188

Human mutation rate revealed

- 100-200 new mutations from one generation to the next;
- One mutation in every 30 million base pairs;

Xue, Y. *et al. Curr. Biol.* (2009) doi:10.1016/j.cub.2009.07.032.

Genomic achievements since the Human Genome Project



ED Green *et al. Nature* **470**, 204-213 (2011) doi:10.1038/nature09764

Dissected OMIM Morbid Map Scorecard

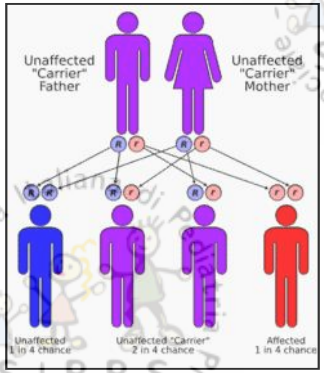
(Updated September 14th, 2016) :

- Single gene disorders and traits: 4,791
- Susceptibility to complex disease or infection: 701
- Somatic cell genetic disease: 205

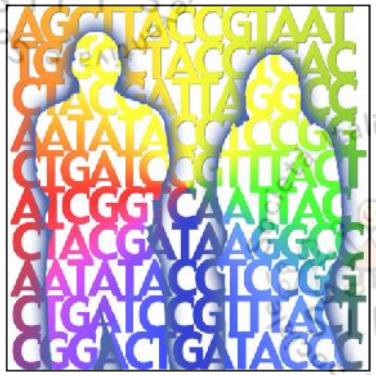
I test genetici

Human Genetic Commission**: A common framework of principles for direct-to-consumer genetic testing services, **2009
http://www.hgc.gov.uk/Client/Content.asp?ContentId=816.

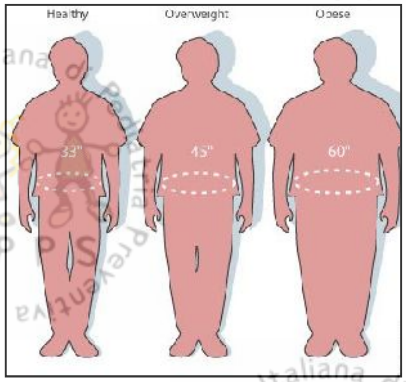
diagnostici



portatori sani

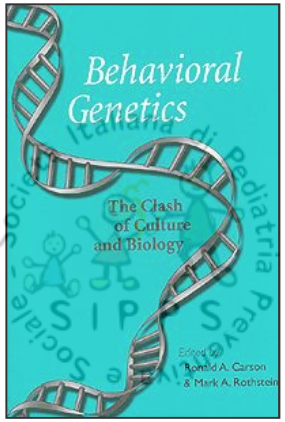


presintomatici

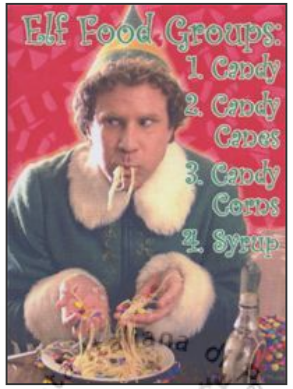


predittivi

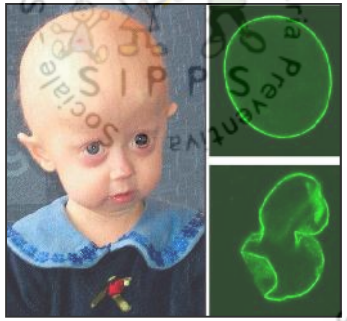
farmacogenetici



test comportamentali e di orientamento sugli stili di vita

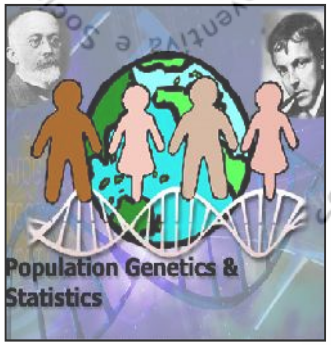


test di nutrigenetica

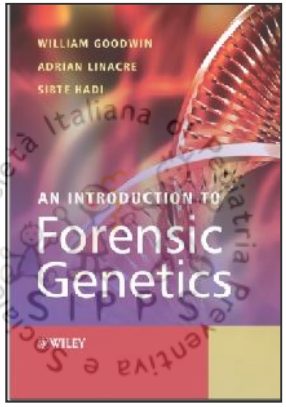


test fenotipici

test per la definizione dei rapporti di parentela

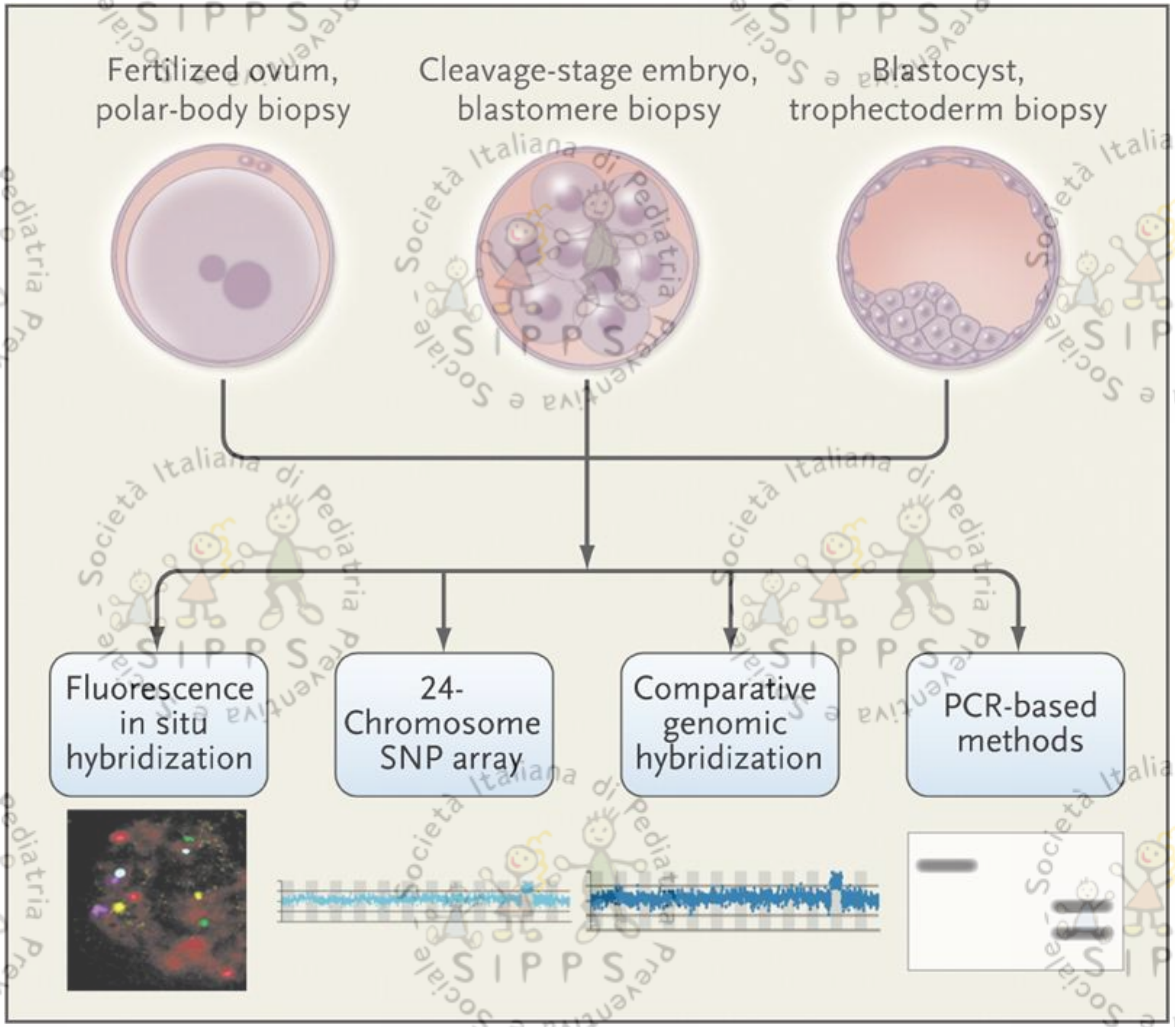


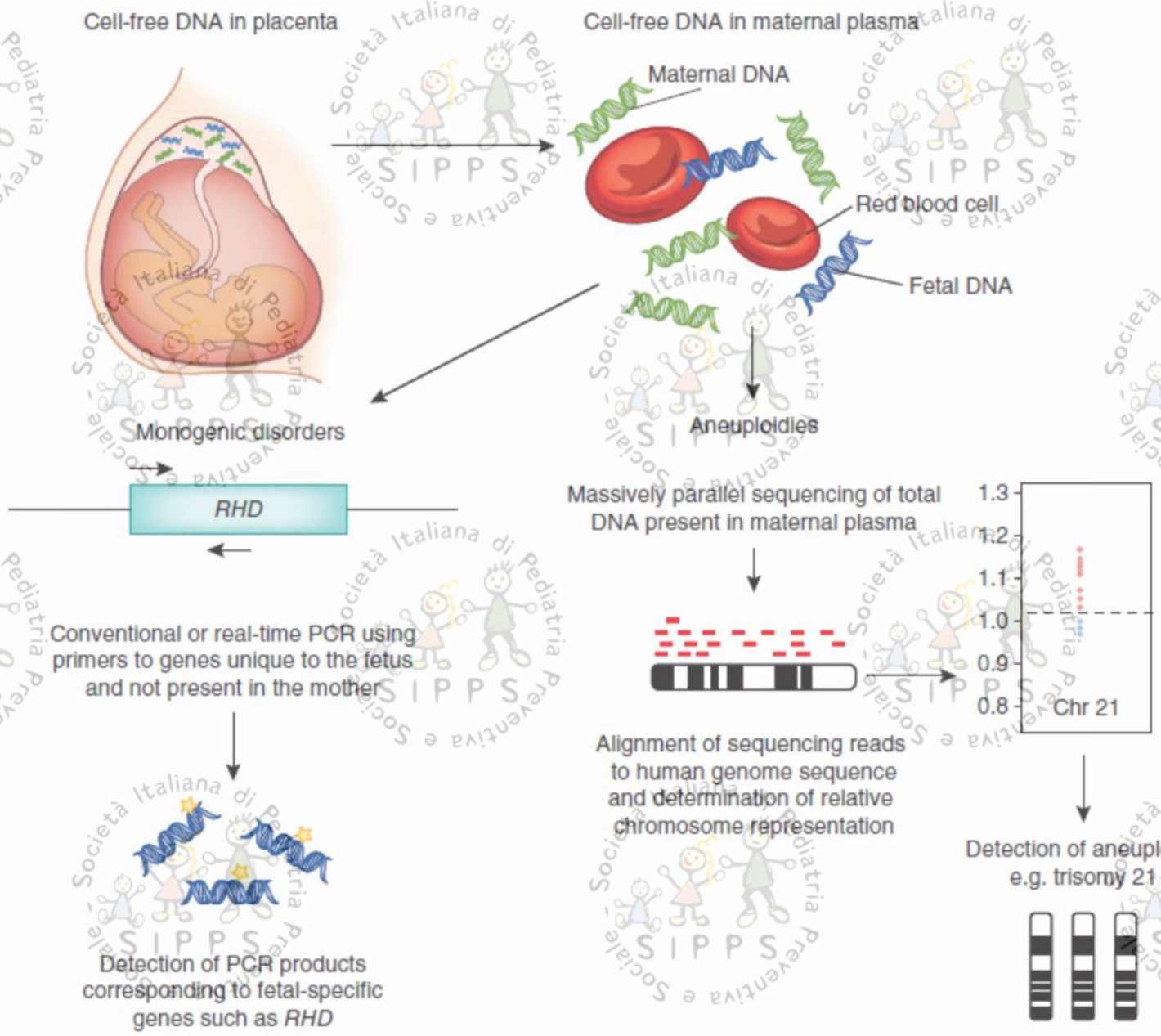
test ancestrali

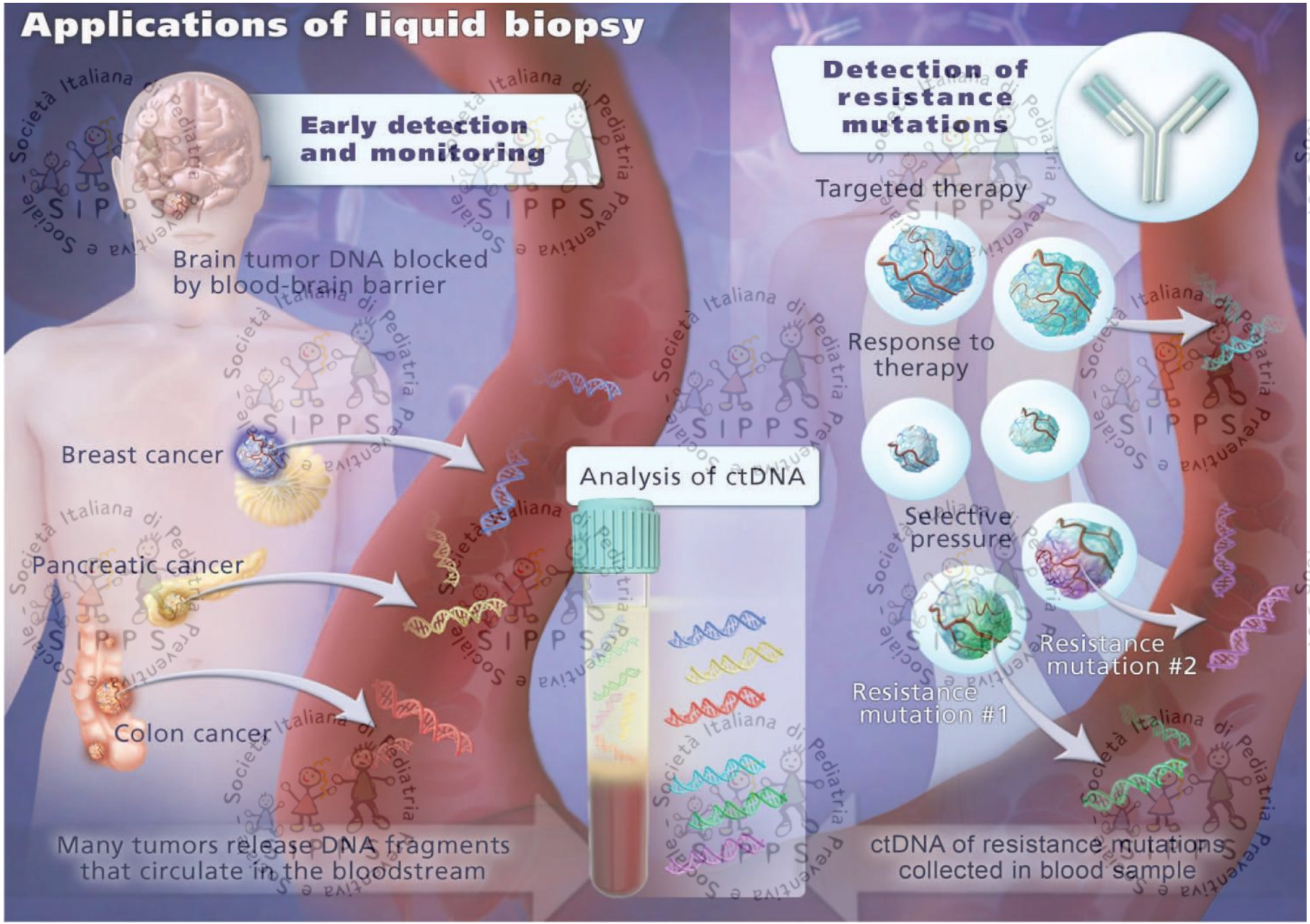


test di compatibilità genetica

Preimplantation Genetic Screening




Cell-free DNA in placenta
Cell-free DNA in maternal plasma
Maternal DNA
Red blood cell
Fetal DNA
Monogenic disorders
Aneuploidies
RHD
Massively parallel sequencing of total DNA present in maternal plasma
1.3
1.2
1.1
1.0
0.9
0.8
Chr 21
Conventional or real-time PCR using primers to genes unique to the fetus and not present in the mother
Alignment of sequencing reads to human genome sequence and determination of relative chromosome representation
Detection of aneuploidy e.g. trisomy 21
Detection of PCR products corresponding to fetal-specific genes such as RHD



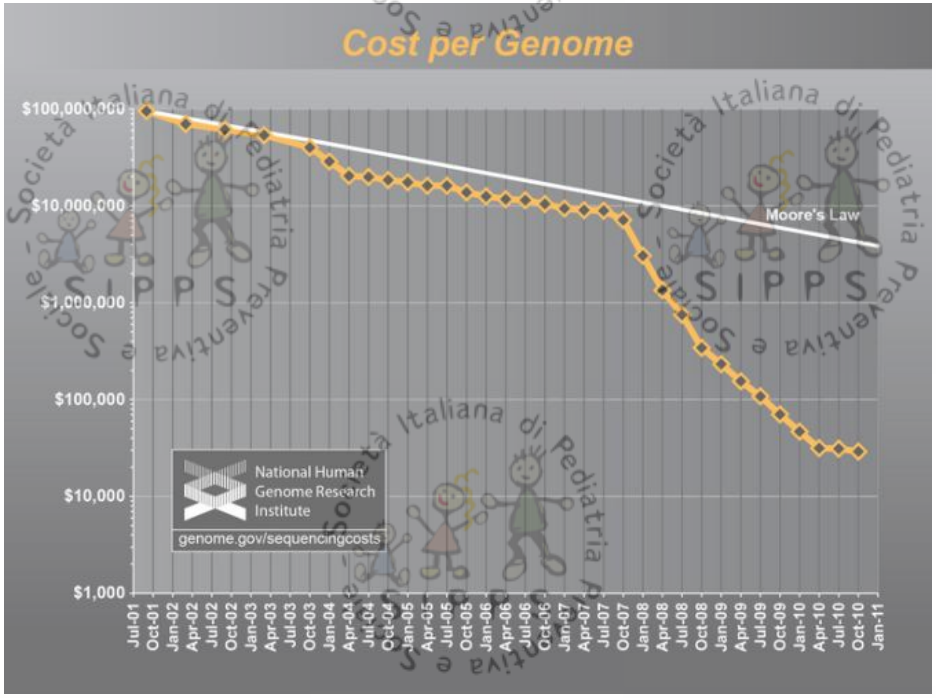
Potential applications of ctDNA.

Chetan Bettegowda *et al.*
Sci Transl Med **6**, 224ra24 (2014);

Sequencing gets cheaper and faster

Cost of one human genome

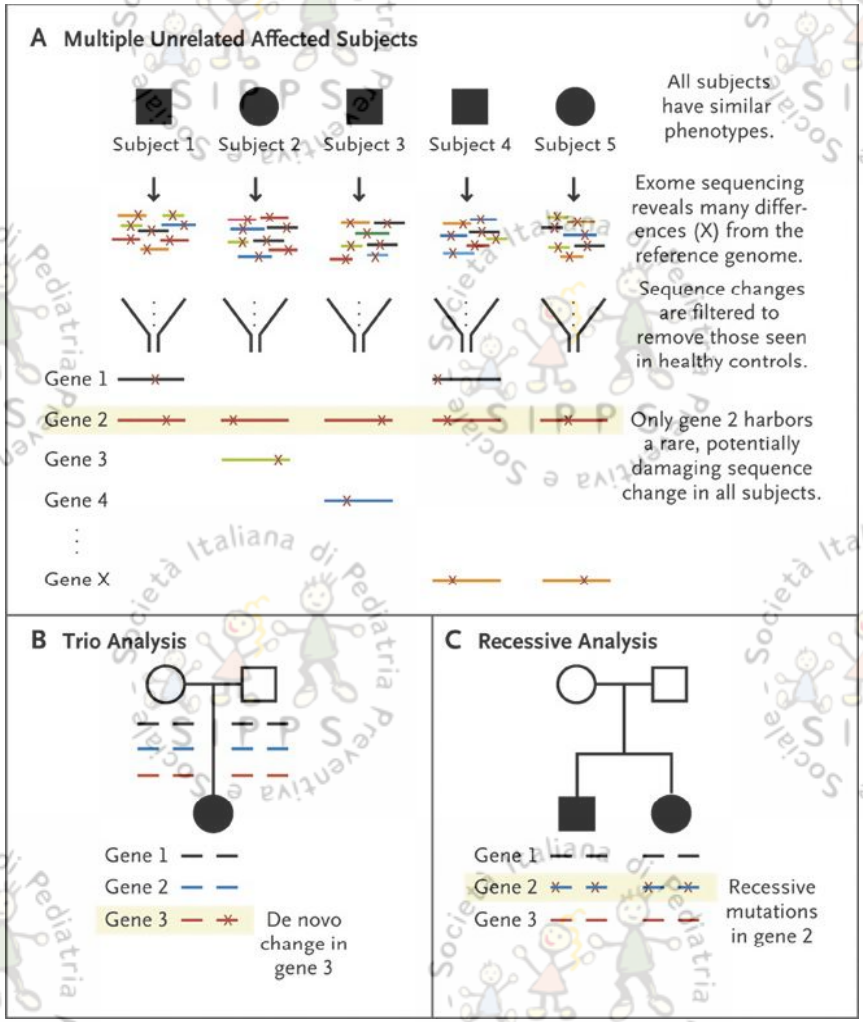
- HGP: $ 3 billion
- 2004: $30,000,000
- 2008: $100,000
- 2010: $10,000
- 2011: $4,000
- 2012-13: $1,000
- ???: $300



courtesy: Batzoglou

Time to sequence one genome: years → days

Massive parallelization.

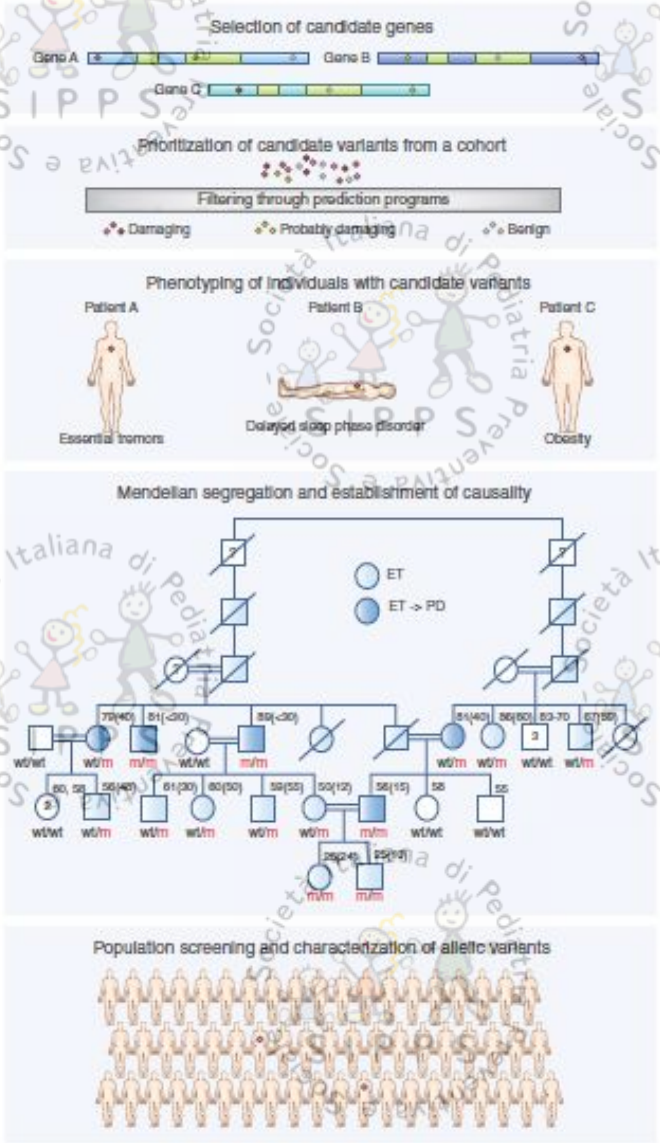


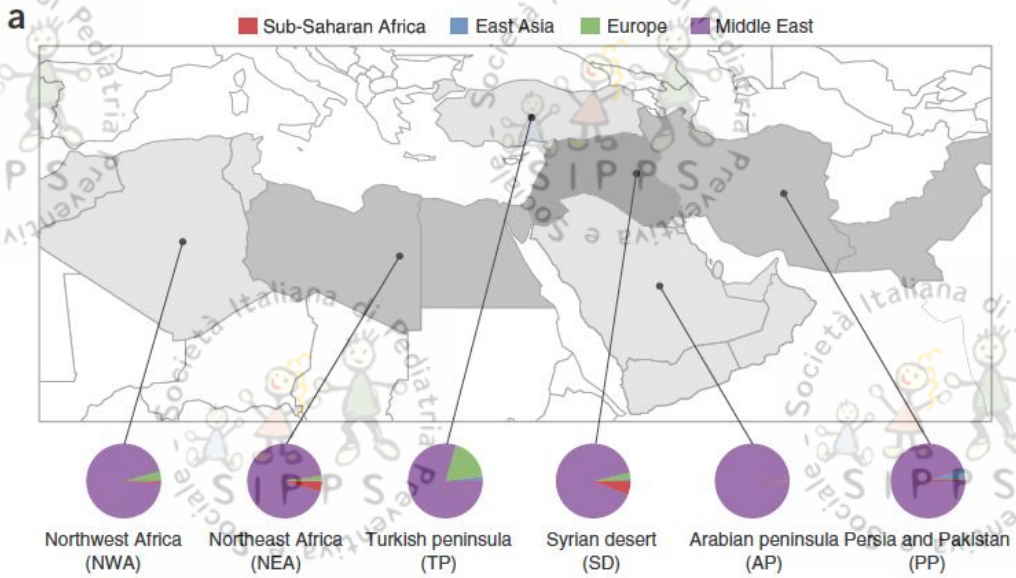
Mefford HC et al. N Engl J Med 2012;366:733-743.

Table 2. Studies Using Massively Parallel Sequencing to Identify Genes Associated with Intellectual Disability and Autism.

Study	Disorder	Presumed Inheritance	Type of Analysis	Genes
Ng et al.[97]	Kabuki syndrome	De novo dominant	Multiple affected	*MLL2*
Hoischen et al.[98]	Schinzel–Giedion syndrome	De novo dominant	Multiple affected	*SETBP1*
Vissers et al.[99]	Nonsyndromic sporadic intellectual disability	De novo dominant	Trio	Multiple
Najmabadi et al.[100]	Recessive intellectual disability	Autosomal recessive, consanguineous families	Targeted recessive	Multiple
Calişkan et al.[101]	Recessive intellectual disability	Autosomal recessive, consanguineous family	Recessive	TECR
O'Roak et al.[102]	Autism	De novo dominant	Trio	*FOXP1, GRIN2B, SCN1A, LAMC3*

Mefford HC et al. N Engl J Med 2012;366:733-743.


Selection of candidate genes
Gene A
Gene B
Gene C
Prioritization of candidate variants from a cohort
Filtering through prediction programs
Damaging
Probably damaging
Benign
Phenotyping of individuals with candidate variants
Patient A
Patient B
Patient C
Essential tremors
Delayed sleep phase disorder
Obesity
Mendelian segregation and establishment of causality
ET
ET -> PD
Population screening and characterization of allelic variants


a
Sub-Saharan Africa
East Asia
Europe
Middle East
Northwest Africa (NWA)
Northeast Africa (NEA)
Turkish peninsula (TP)
Syrian desert (SD)
Arabian peninsula (AP)
Persia and Pakistan (PP)

Pathogenic Variants for Mendelian and Complex Traits in Exomes of 6,517 European and African Americans

- 70 genes analyzed by ES:
  - identified 10,789 variants
  - 399 validated pathogenic variants.
- The mean number of risk alleles per individual was 15.3;
- Every individual had at least five known PGx alleles;
- 99% of individuals had at least one ARMD risk allele;
- 45% of individuals were carriers for at least one pathogenic NBS allele;
- The carrier burden for severe recessive childhood disorders was 0.57;

- Our results demonstrate that risk alleles of potential clinical utility for both Mendelian and complex traits are detectable in every individual.

….Many parents of children in pediatric genomic research indicated a strong desire to receive a broader range of results ……

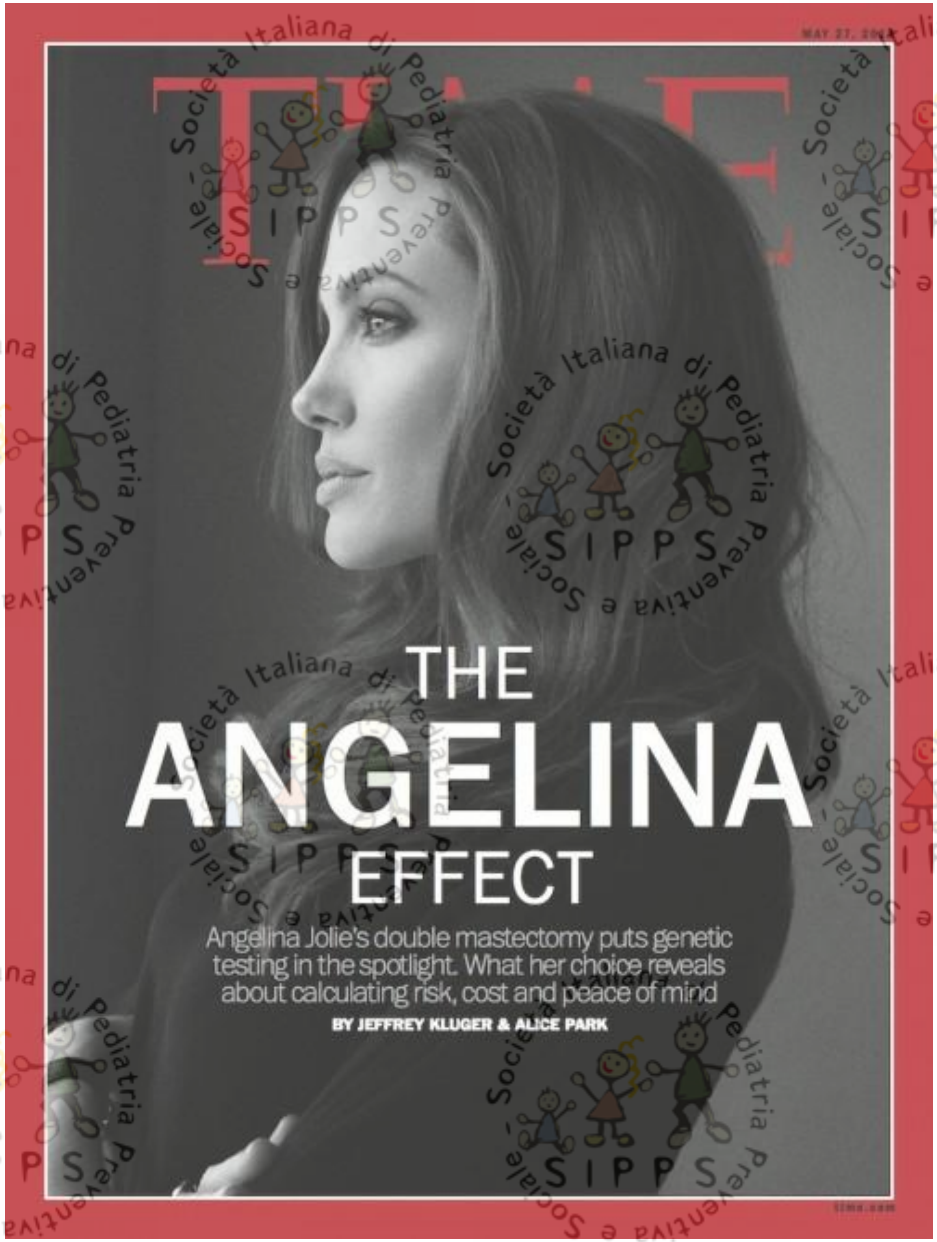
- ✓ **No difference was found in results between participants with cancer and those with orphan diseases.**
- ✓ **92% indicated that genomic research for childhood-onset conditions should occur.**
- ✓ **The majority wanted incidental results predicting susceptibility even to untreatable fatal conditions (83%), to multiple conditions (87%), or to those with uncertain impact (70%).**
- ✓ **Most felt sibling genomic results showing serious conditions, whether treatable (93%) or not (88%), and/or results discovered after death of the proband should be shared with family (74%).**

Genetics in Medicine (2014) **16**, 633–640
doi:10.1038/gim.2013.201

l’arrivo dei “non pazienti”….

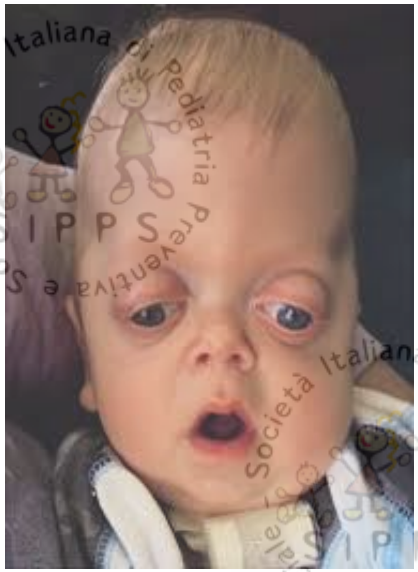
- “sono persone che non presentano sintomi evidenti e che condividono predisposizioni genetiche, vivendo nell’attesa probabilistica di comparsa di qualche segno di malattia, organizzando la loro vita in funzione delle visite mediche o delle analisi di laboratorio, e che poi finiscono per sentirsi ammalati o addirittura che sviluppano sintomi psicosomatici”.

Novelli G., Quotidiano on line di informazione sanitaria
Martedì 14 APRILE 2015

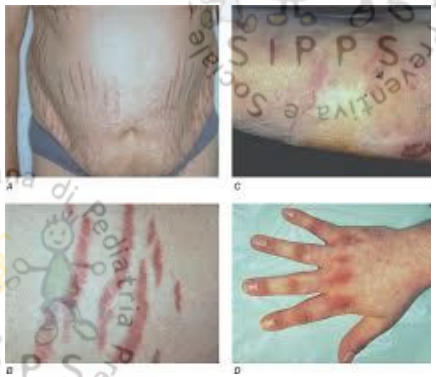

TIME
THE
ANGELINA
EFFECT
Angelina Jolie's double mastectomy puts genetic testing in the spotlight. What her choice reveals about calculating risk, cost and peace of mind
BY JEFFREY KLUGER & ALICE PARK

Genetic Superheroes?

Perhaps you or someone you know is a genetic superhero and simply doesn't know it yet.

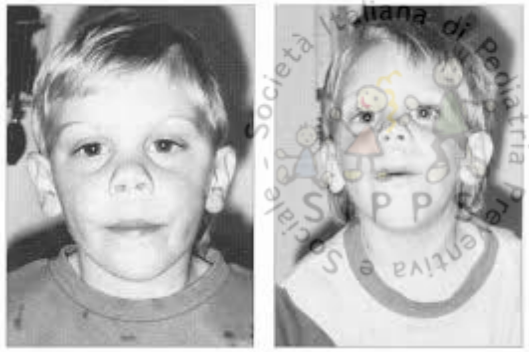


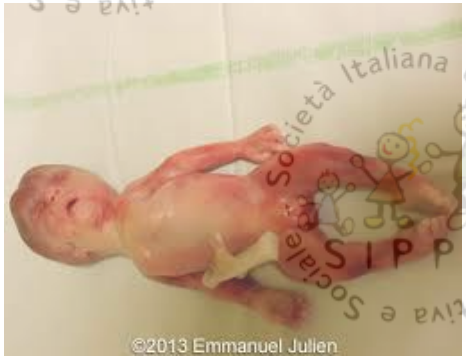
Superheroes of disease resistance



NATURE BIOTECHNOLOGY, 2016

870 mutations, causing 580 mendelian conditions





The Escapers

- PCSK9, loss of function protected against high lipid levels
- CCR5/HIV, resistence to HIV infection
- ZNT8, protects even obese from diabetes
- PLS3, rescue the spinal muscular atrophy patients in SMN deleted
- Stabilizing Effects of Interruptions on Trinucleotide Repeat Expansions

Genoma Personale- carta del DNA ?

Stephen Quake

Sequenziamento del genoma

- Alto rischio genetico di Infarto del Miocardio.
- Alto rischio genetico di sviluppare Diabete di tipo 2.
- Alto rischio genetico di sviluppare cancro alle paratiroidi e iperparatiroidismo.
- Alto rischio di obesità.
- Rare varianti in tre geni (TMEM43, DSP, e MYBPC3), associati a morte cardiaca improvvisa.
- 63 variazioni che modulano la risposta ai farmaci

SANO

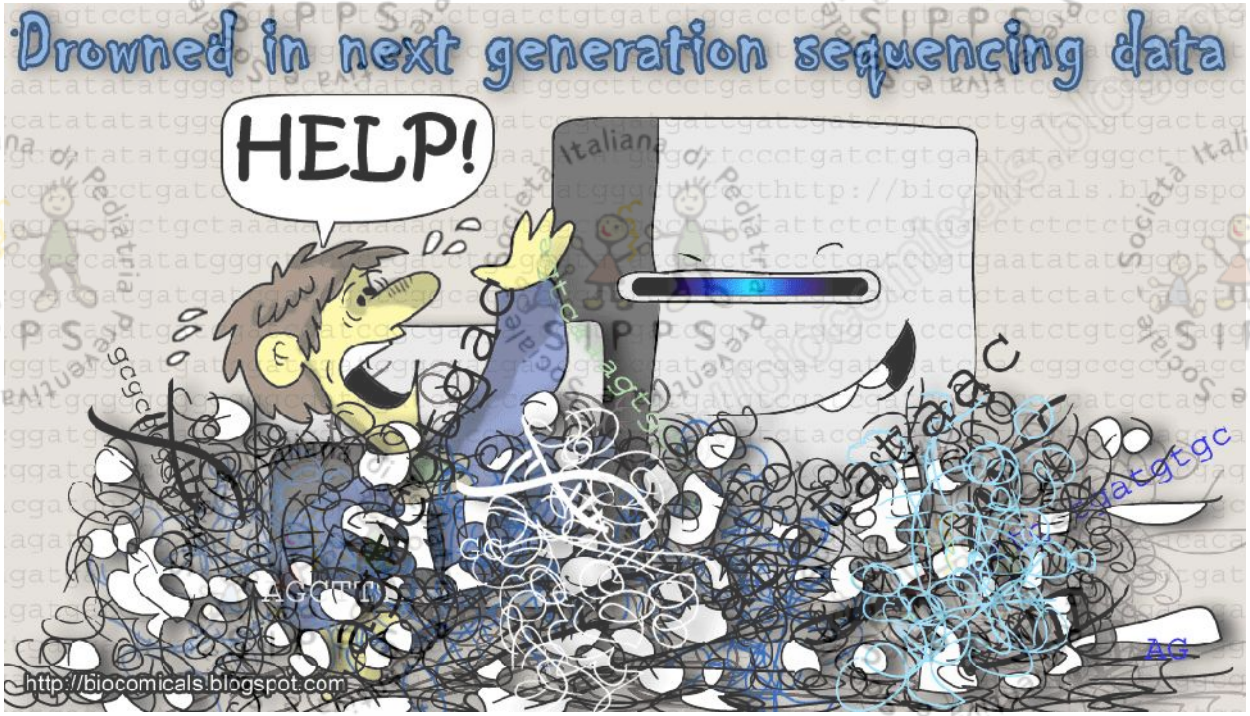
Basso rischio genetico di sviluppare l' Alzheimer

ANCORA SANO ?

Ashley *et al.*, Lancet 2010

Nuove tecnologie = generazione di grandi quantità di dati.

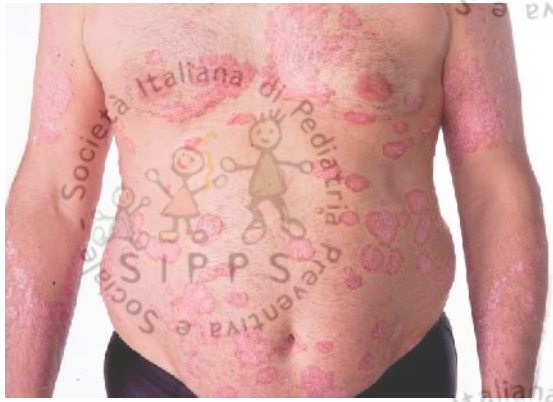
In questo ambito si collocano anche i cosiddetti “incidental findings”.

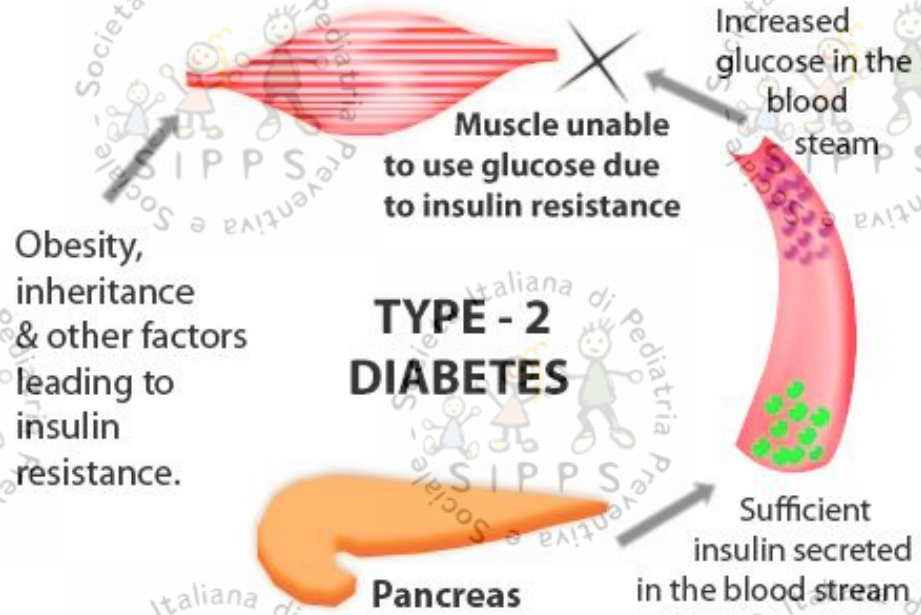


Phenotype determination

- **1. Different Genes/Mutations**
- **2. Different Populations**
- **3. Individual “genometype”**
- **4. Different Environments**

Psoriasis, Type 2 Diabetes Mellitus, and Obesity Weighing the Evidence





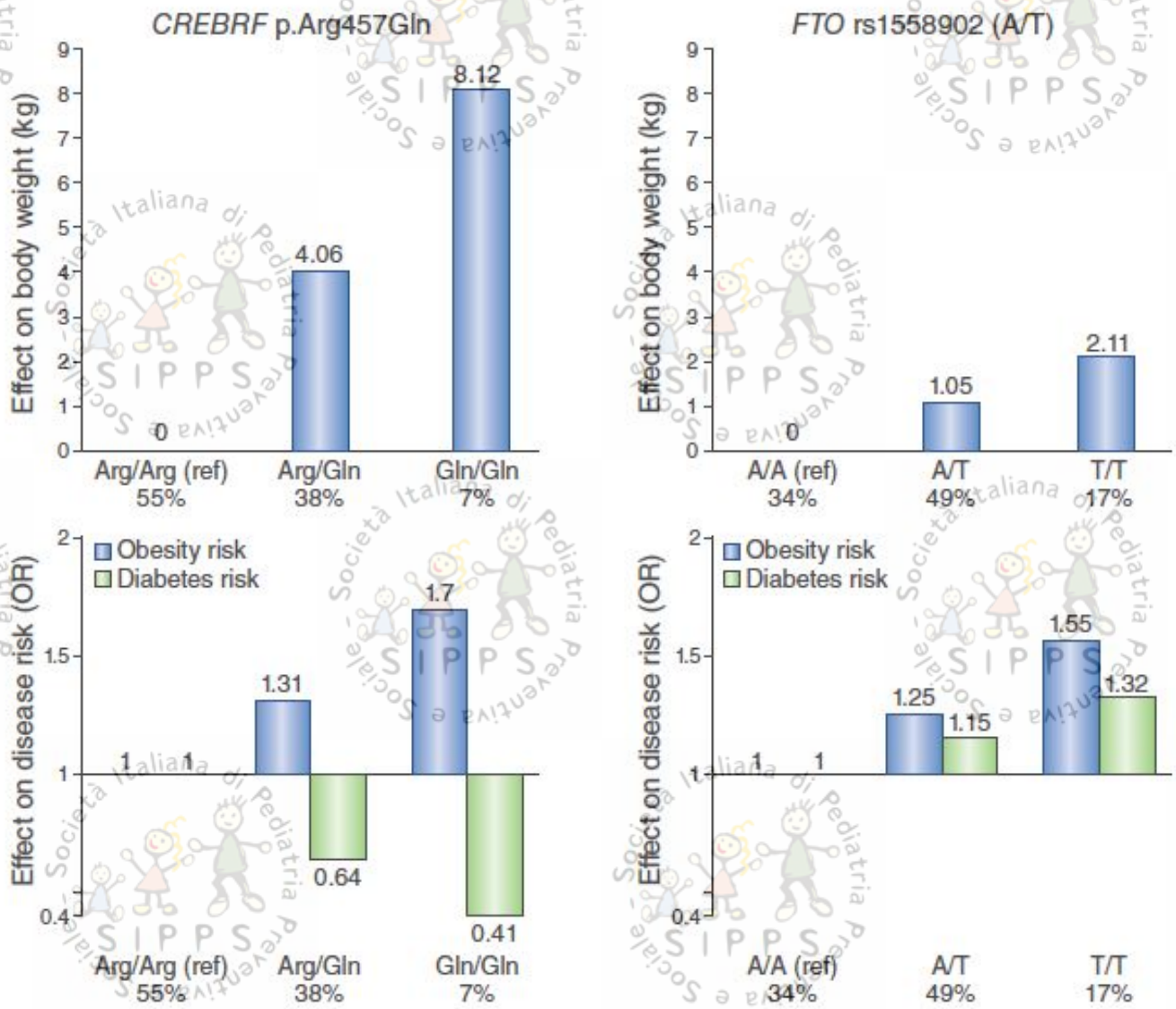
Gelfald, 2016

George Henderson @puddleg · Jul 25

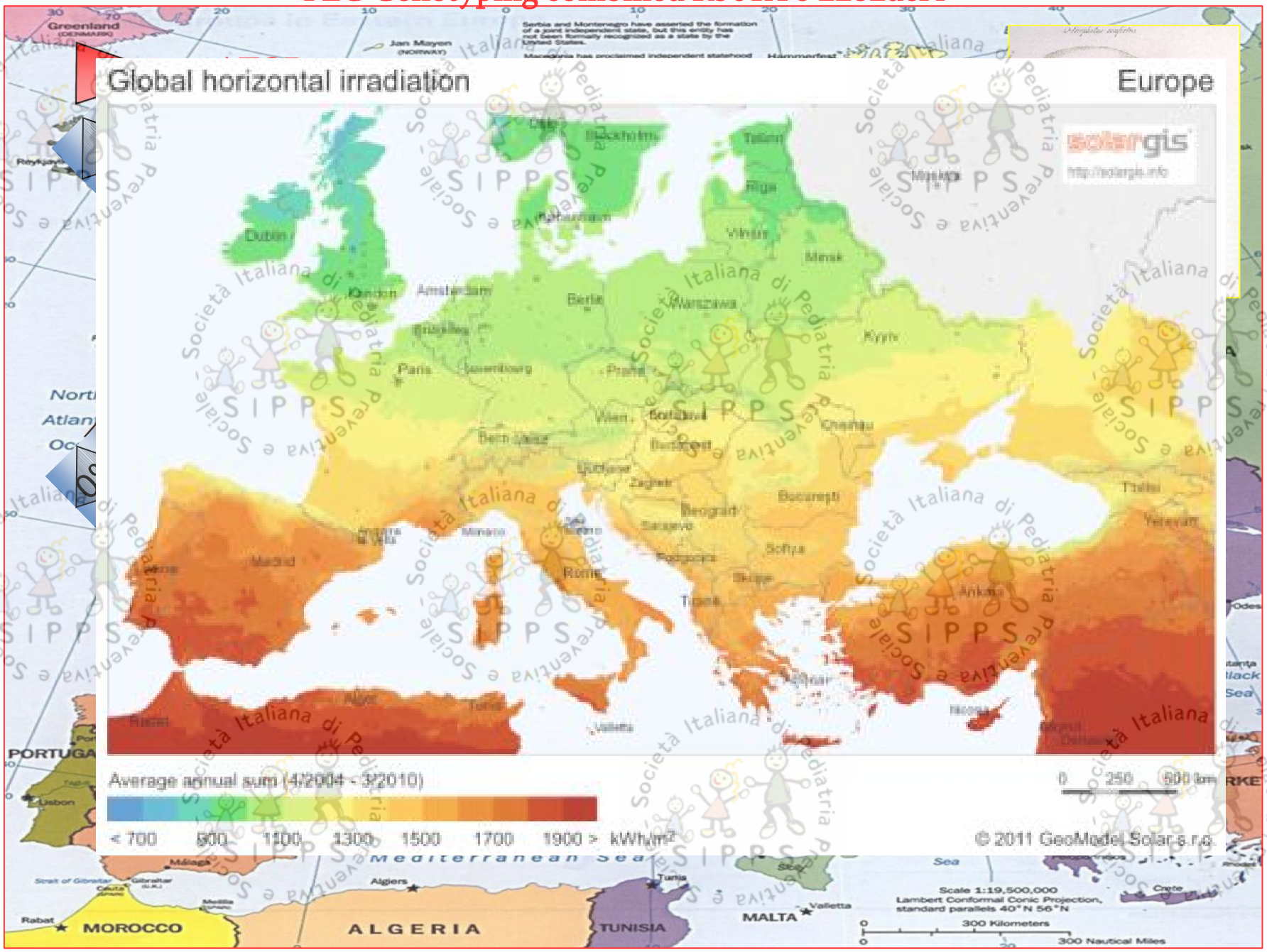
Samoan men 1930, before Western food (refined starch, sugar and oil) dominated the Samoan diet. Same genes.

Samoan today

Thrifty gene or drifty gene?

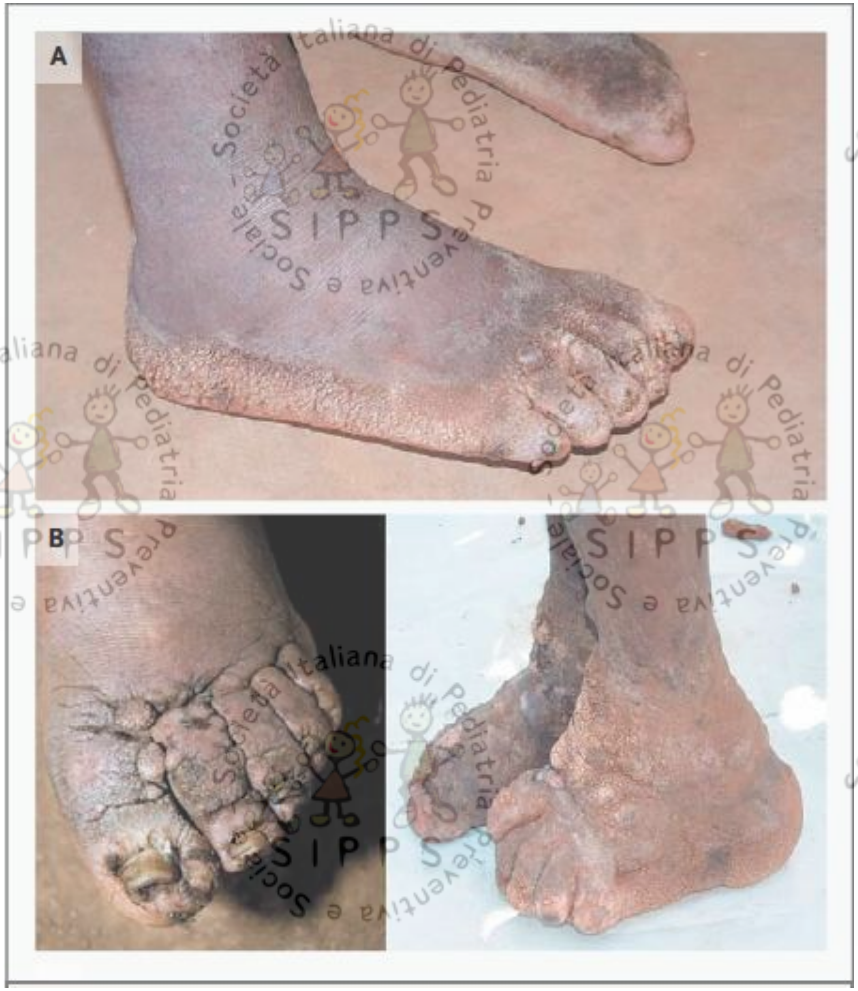


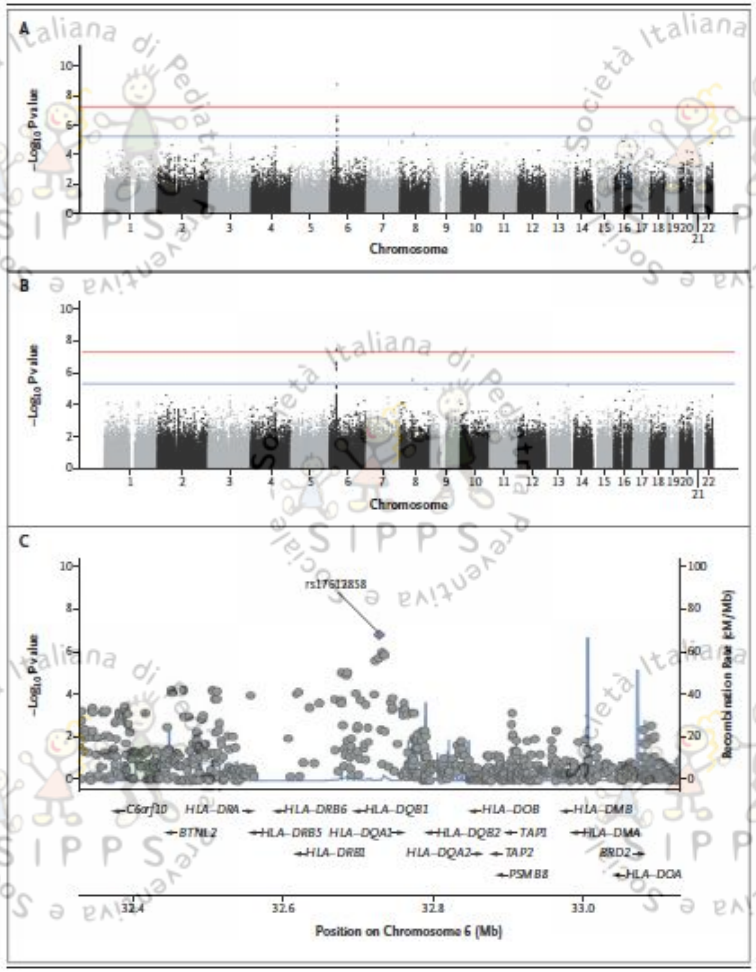
FLG Genotyping combined R501X e 2282del4



a causa di siccità, carestie e scarsità di acqua potabile, soffrono la fame e la sete, e dove un agricoltore su 20 si ammala di podoconiosi

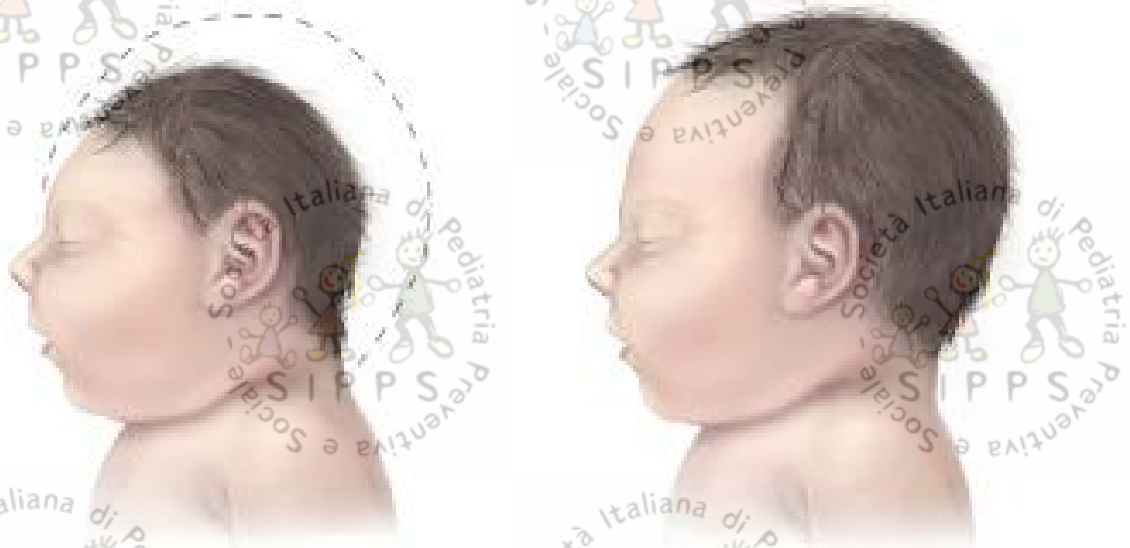
Wolayta


A
B


A
B
C
−Log10 P value
Chromosome
rs17612858
Recombination Rate (cM/Mb)
C6orf10
HLA-DRA
HLA-DRB6
HLA-DQB1
HLA-DOB
HLA-DMB
BTNL2
HLA-DRB5
HLA-DQA1
HLA-DQB2
TAP1
HLA-DMA
HLA-DRB1
HLA-DQA2
TAP2
BRD2
PSMB8
HLA-DOA
Position on Chromosome 6 (Mb)

CDC: Zika Causes Microcephaly

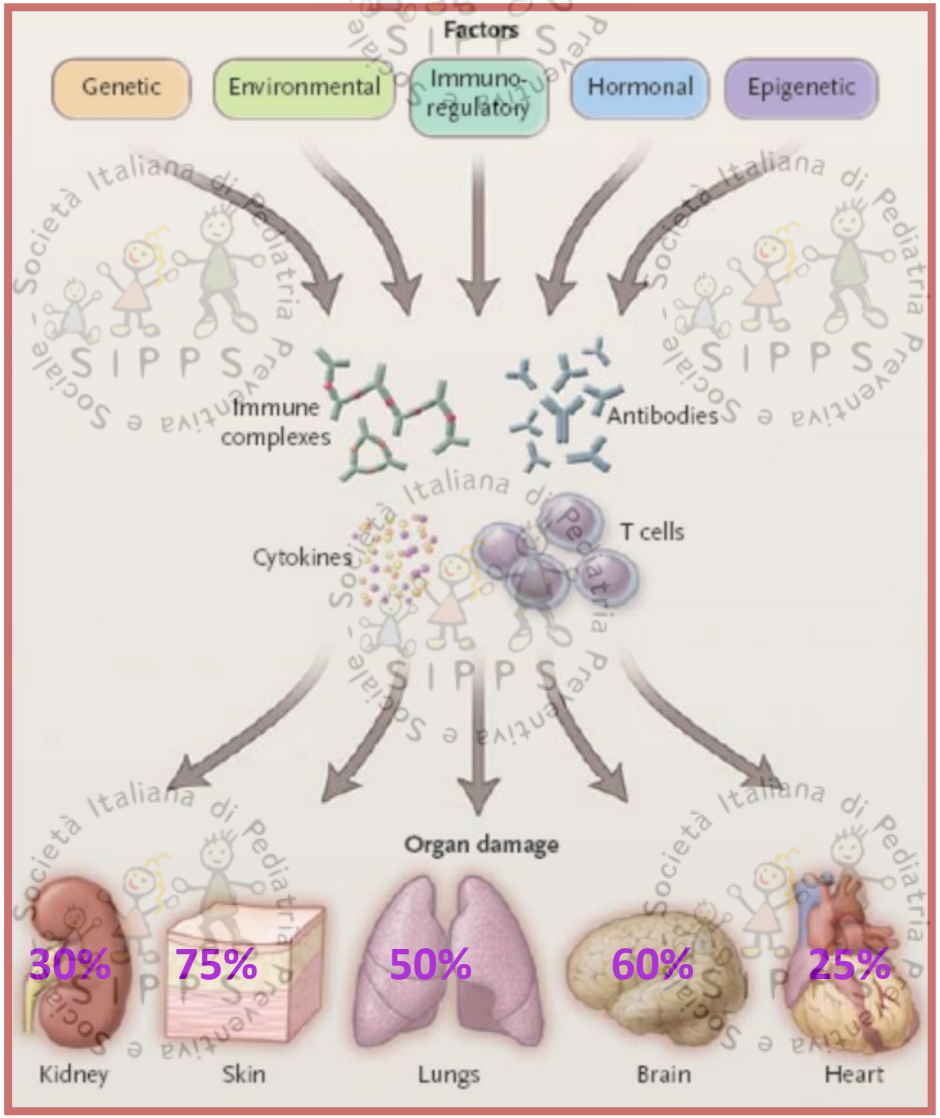
The virus is also to blame for other birth defects, the US Centers for Disease Control and Prevention concludes.



Lupus Eritematoso Sistemico (LES)

- ✓ Malattia infiammatoria cronica, di natura autoimmune, ad eziologia complessa.

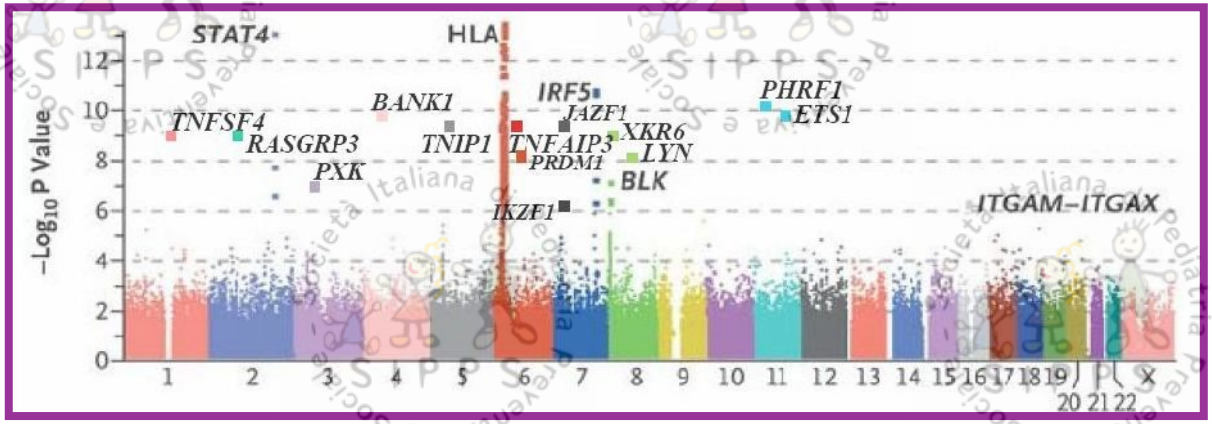
- ✓ Il LES è caratterizzato dall'interessamento, anche non contemporaneo, di numerosi organi ed apparati e da un'importante disregolazione del sistema immunitario.

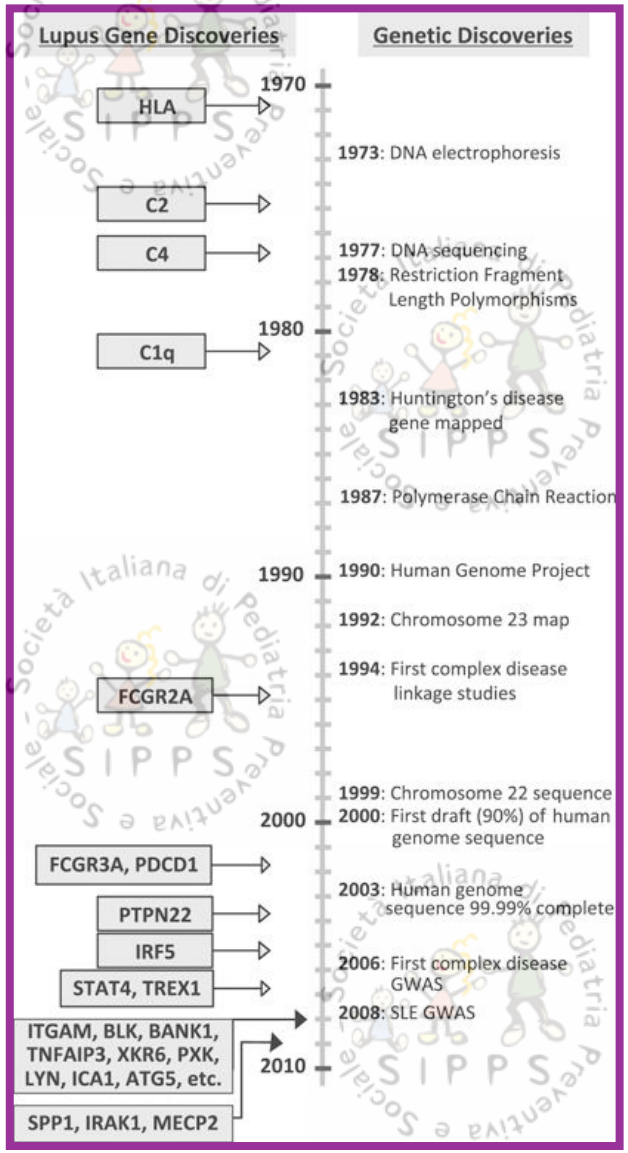


Individuazione fattori genetici nel LES

Identificati diverse decine loci coinvolti nel meccanismo patogenetico del LES

- Analisi di linkage
- Studi di associazione su geni candidati
- Studi di Genome-Wide Analysis (GWAS)

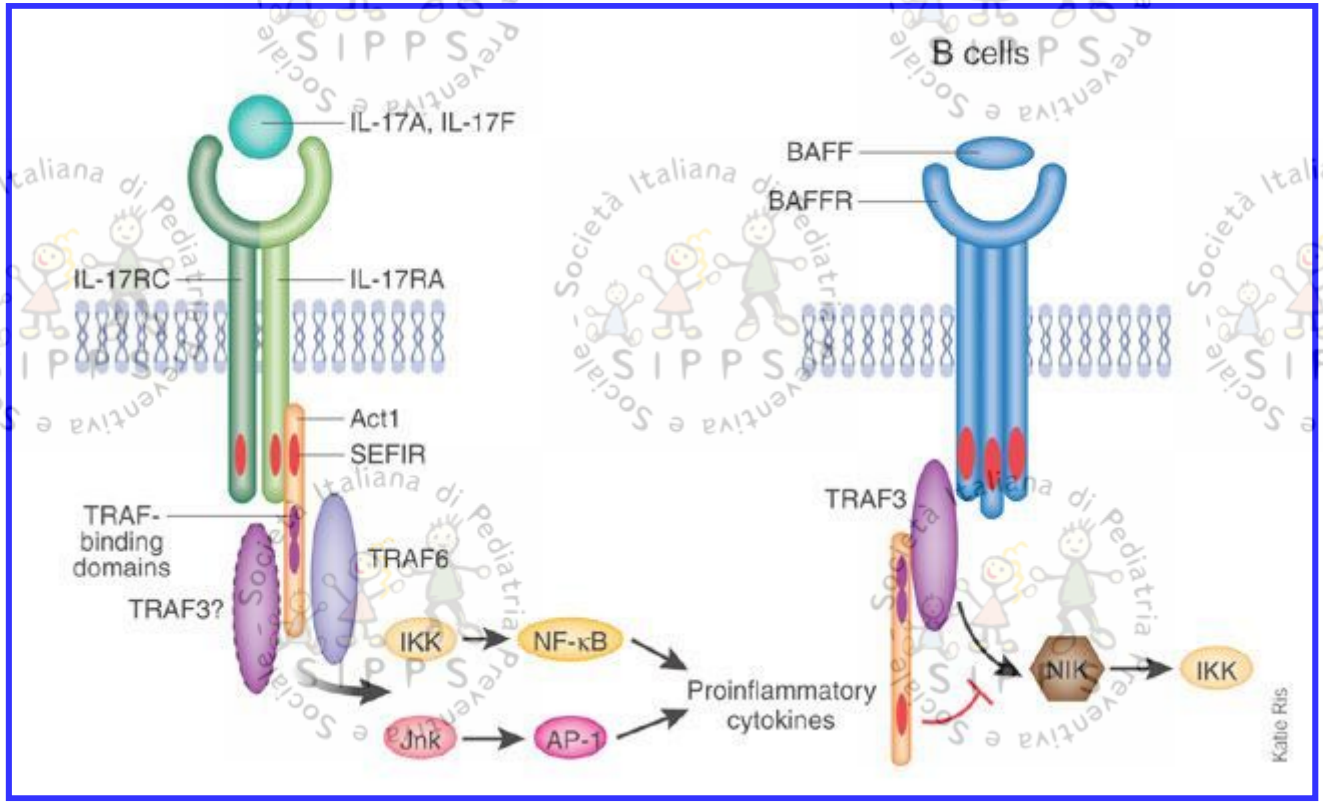


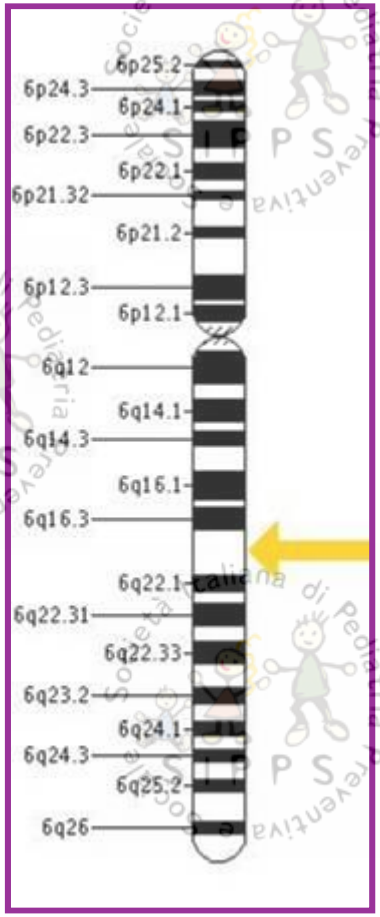


TRAF3IP2

TRAF3IP2 codifica per ACT1 che funge da:

- regolatore positivo dell'attivazione di NF-κb dipendente da IL-17
- regolatore negativo dell'immunità umorale.

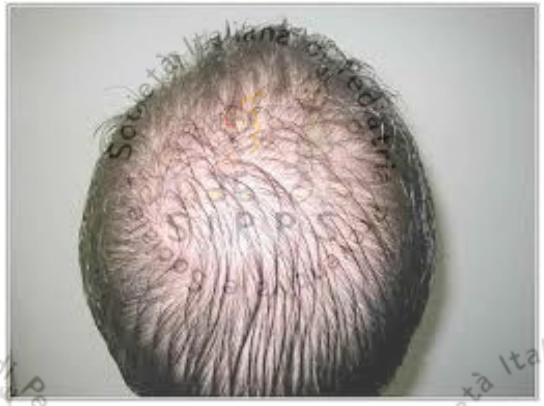


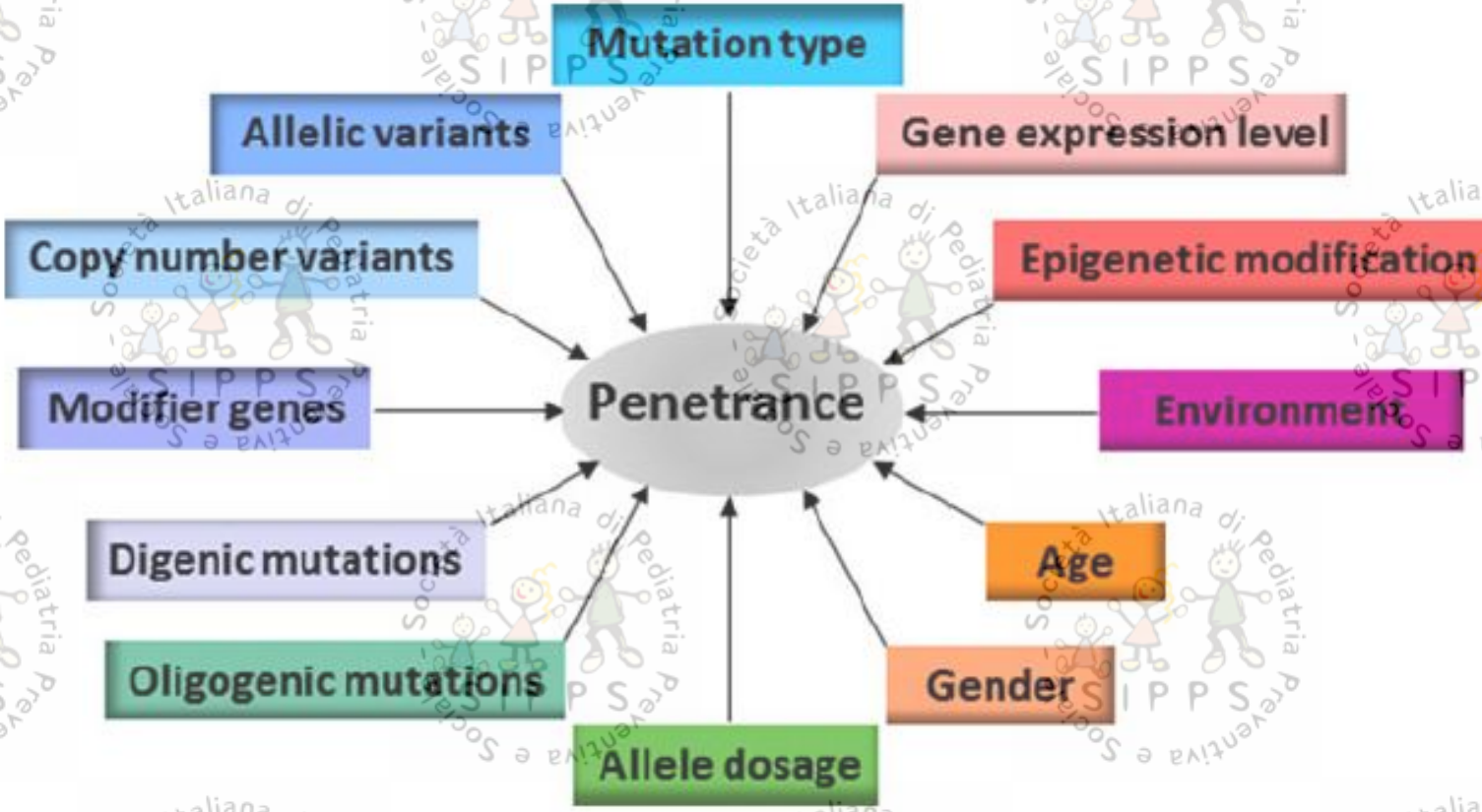


- ✓ I nostri dati mostrano per la prima volta il contributo di TRAF3IP2 nella suscettibilità al LES: il gene TRAF3IP2 conferisce un rischio aumentato di circa 1,7 volte di sviluppare il LES (OR= 1.7; 95% C.I. 1.08-2.72).
- ✓ Soprattutto che le varianti di questo gene contribuiscono a sviluppare Pericardite nei malati LES (OR=2.59; 95% C.I. 1.39-4.80; OR=2.38; 95% C.I. 1.19-4.79; OR=2.44; 95% C.I. 1.21-4.93 per gli SNPs rs33980500, rs13190932 e rs13193677 rispettivamente).
- ✓ Questo risultato conferma che varianti comuni nei geni coinvolti nella risposta immunitaria sono importanti sia nella suscettibilità al LES che nella modulazione del fenotipo malattia.

Genetic Connections Among Human Traits

A study identifies genetic variants that are linked to multiple phenotypes




Mutation type
Allelic variants
Gene expression level
Copy number variants
Epigenetic modification
Modifier genes
Penetrance
Environment
Digenic mutations
Age
Oligogenic mutations
Gender
Allele dosage

Simple but important questions in medicine

Why some individuals get sick more easily?

Why is treatment succesful only in some individuals?

Why are some individuals more prone to adverse effects?

“If you have cancer in 2014, the first thing we do is a genetic test for discovering drivers mutations…..”

Nature 11 September 2014

“There are more paths to developing tumours than there are stars in the sky”

R. Weinberg

Galaxy 200 billion
Universe 3,000 million billion

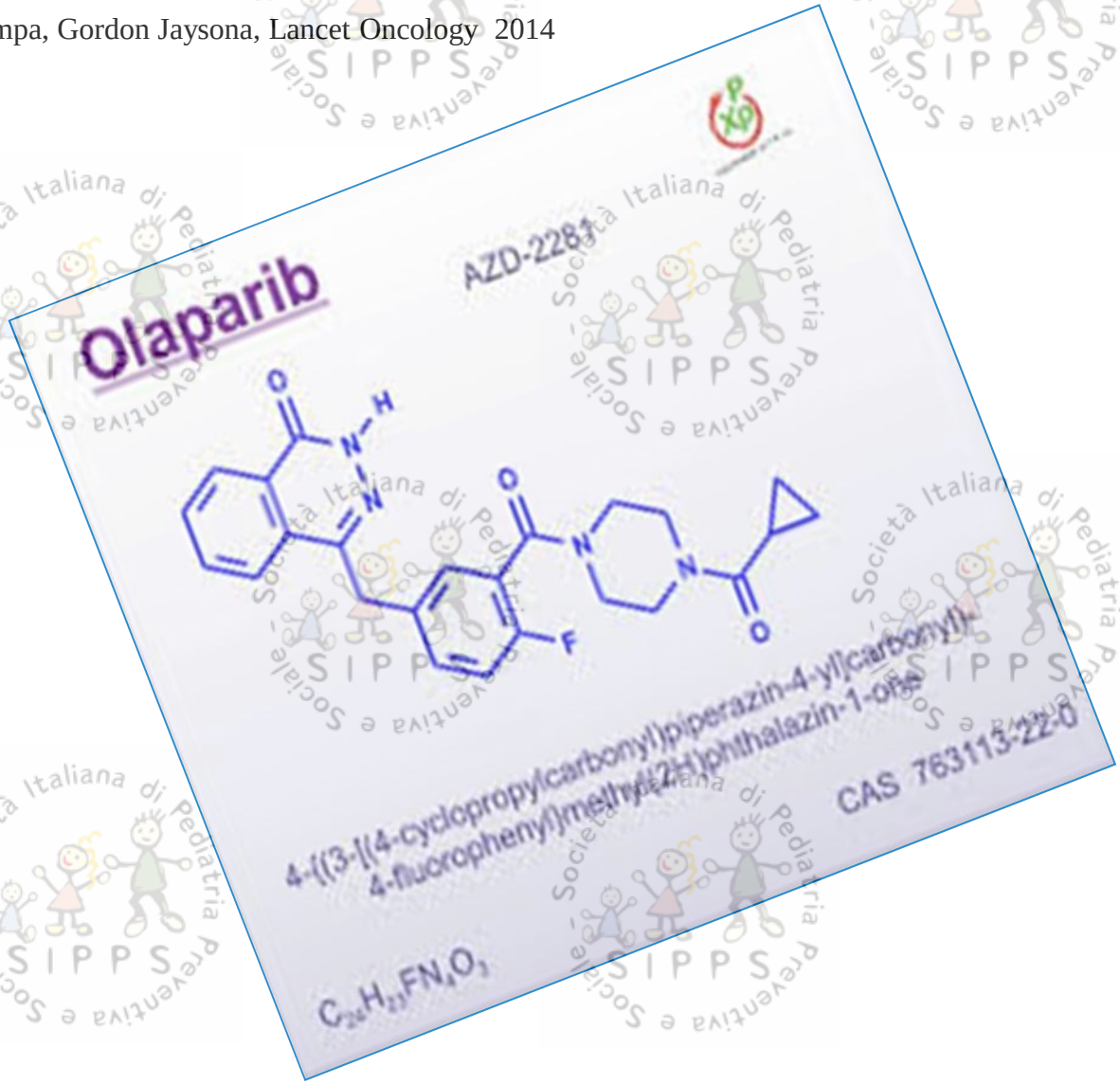
CML, APL, Her-2
(Ovarian, NB, Thyroid)

K-ras, p53, p16, smad4
(Breast/Pancreas/Colon)

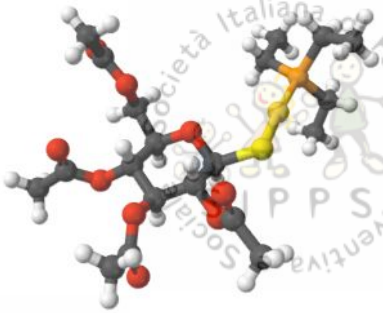
EGFR, ALK, mTOR)
(Melanoma,
/Lung/Stomach)

PARP inhibitors in BRCA mutation-associated ovarian cancer

Andrew Clampa, Gordon Jaysona, Lancet Oncology 2014



Ovarian cancer with BRCA1 mutation may be treatable with arthritis drug



- Auranofin, a thioredoxin reductase inhibitor
- Auranofin exerts its cytotoxic activity by increasing the production of reactive oxygen species(ROS).

BM-based indications (oncology)

Table 1 | Drugs licensed in the European Union with biomarker-restricted indications

Medicinal product	Indication	Biomarker
Cetuximab	Colorectal cancer	EGFR positive* *KRAS* wild type‡
Catumaxomab	Malignant ascites	EpCam positive*
Dasatinib	CML	BCR–ABL§
Erlotinib	NSCLC	EGFR positive* EGFR TK mutation positive§
Gefitinib	NSCLC	EGFR TK mutation positive‡
^{90}Y-Ibritumomab	Follicular lymphoma	CD20*
Imatinib	CML Myelodysplastic or proliferative disease Hypereosinophilic syndrome Gastrointestinal stromal tumors	BCR–ABL§ *PDGFR* gene re-arrangements§ *FIP1L1–PDGFR* rearrangement§ c-Kit§
Lapatinib	Breast cancer	HER2 overexpression‖
Nilotinib	CML	BCR–ABL§
Panitumumab	Colorectal cancer	EGFR* *KRAS* wild type‡
Rituximab	Non-Hodgkin lymphoma	CD20*
Trastuzumab	Breast cancer	HER2 overexpression‡
Vemurafenib	Malignant melanoma	BRAF V600§

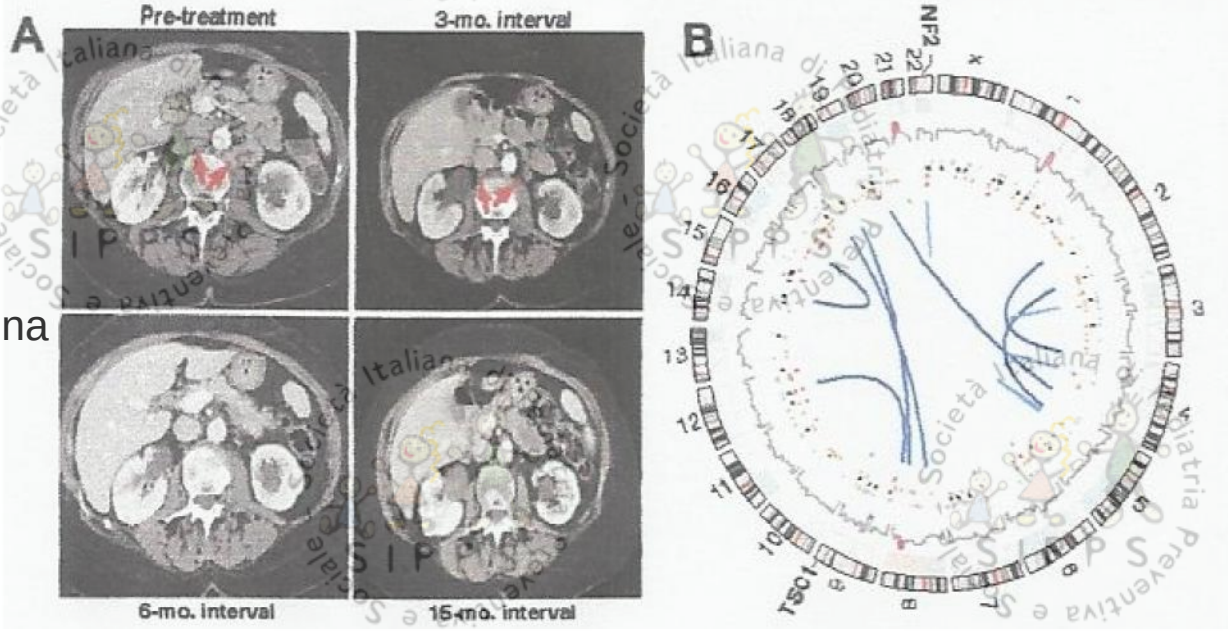
*Drug development based on a monoclonal antibody and antigen expression or small molecules with a defined target. ‡Biomarker identified during prospective or retrospective stratified drug development. §Drug development driven by previous identification of the target. ‖Follow on after the development of trastuzumab. Abbreviations: CML, chronic myeloid leukemia; NSCLC, non-small-cell lung cancer; TK, tyrosine kinase.

Genome Sequencing Identifies a Basis for Everolimus Sensitivity

Gopa Iyer,* Aphrothiti J. Hanrahan, Matthew I. Milowsky, Hikmat Al-Ahmadie, Sasinya N. Scott, Manickam Janakiraman, Mono Pirun, Chris Sander, Nicholas D. Socci, Irina Ostrovnaya, Agnes Viale, Adriana Heguy, Luke Peng, Timothy A. Chan, Bernard Bochner, Dean F. Bajorin, Michael F. Berger, Barry S. Taylor,† David B. Solit†

www.sciencemag.org SCIENCE VOL 338 12 OCTOBER 2012

In un paziente sensibile a everolimus, un inibitore del complesso MTORC1, il sequenziamento
del genoma tumorale ha rivelato una mutazione nel gene *TSC1* e una mutazione nel gene *NF2.*



Drug repositioning for personalized medicine

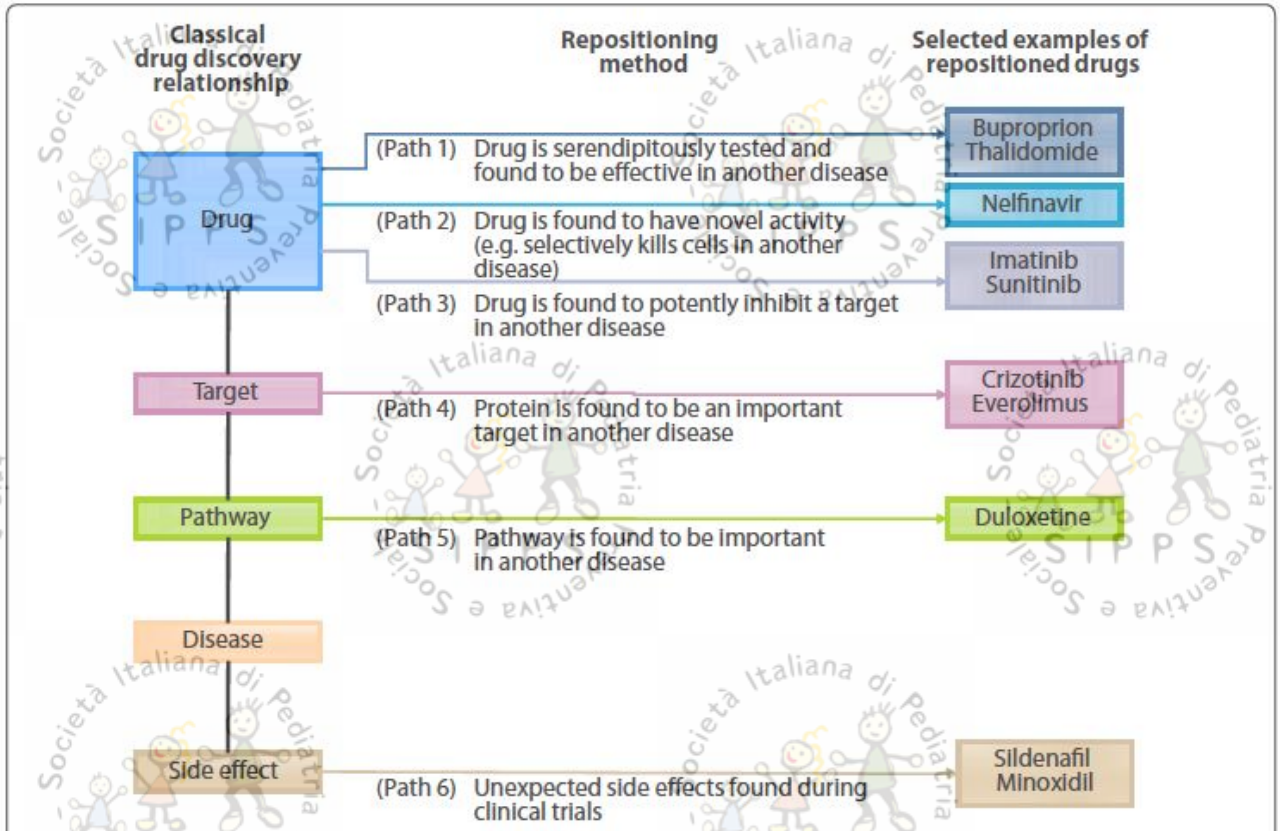
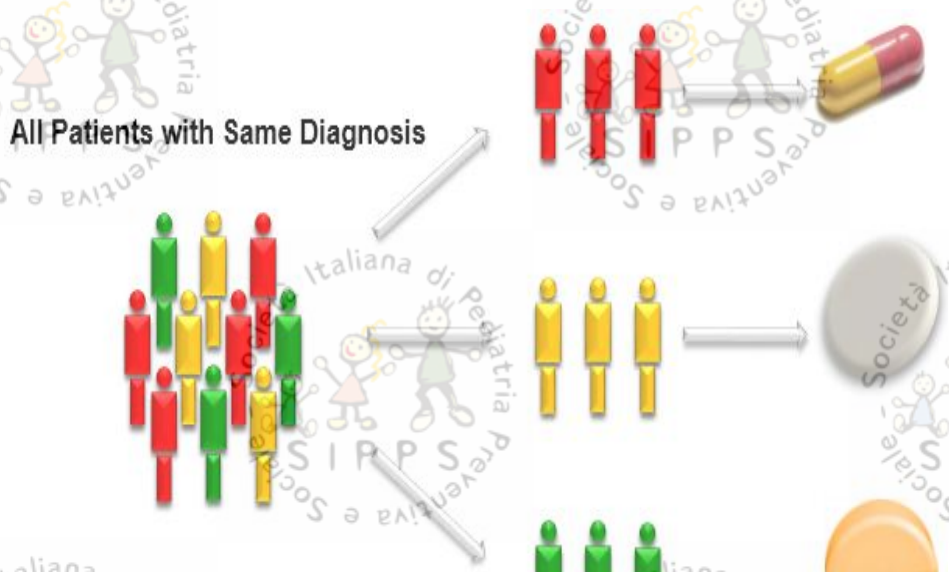


Figure 1. Potential avenues of drug repositioning. Most repositioned drugs so far have been discovered through serendipitous treatment or unexpected side effects observed during clinical trials (path 1, path 6). More rational approaches to the identification of drug repositioning candidates involve finding existing drugs that can modulate specific disease phenotypes (path 2), finding new drug-target interactions (path 3), finding new roles for existing targets (path 4), or finding new pathways in disease (path 5). One or two examples of successfully repositioned drugs are listed for each method.

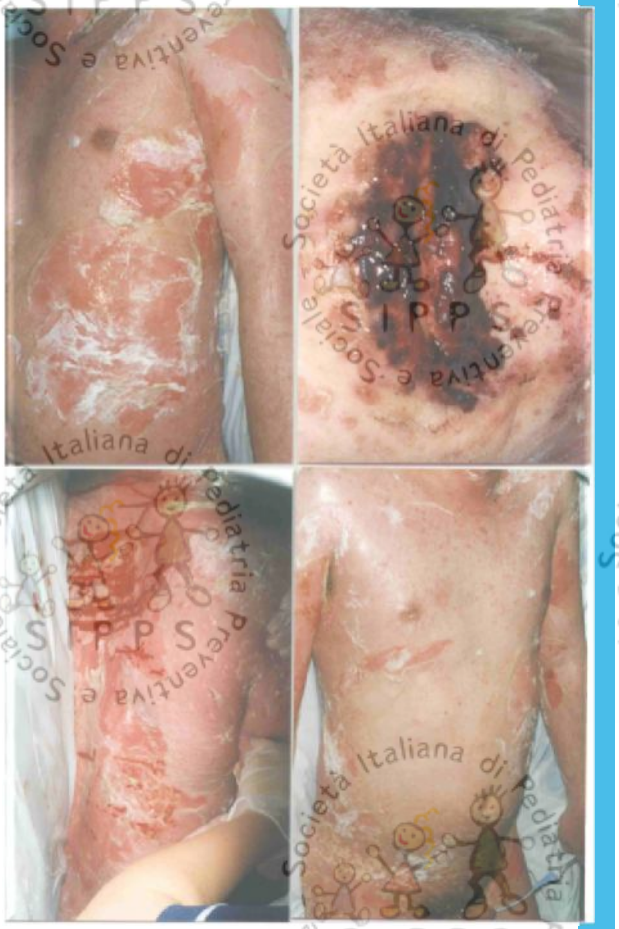
Precise medicine:
"The right treatment for the right person at the right time."



Delayed Hypersensitivity Reactions (4)

Toxic Epidermal Necrolysis

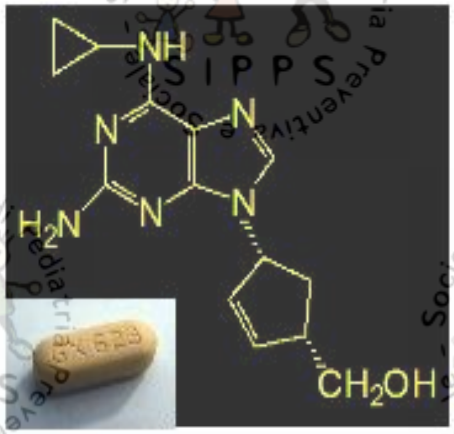
- >30% of skin blistered
- Two mucous membranes involved
- 30% mortality



Abacavir Hypersensitivity: A Paradigm for Translational Pharmacogenetics

MRC Centre for Drug Safety Science

Italy
Pretesting 6%
Post testing 0%



Clinical genotype

Association with HLA-B*5701

Cost-effectiveness analysis of HLA *B*5701* genotyping in preventing abacavir hypersensitivity
Dyfrig A. Hughes[a], F. Javier Vilar[b], Charlotte C. Ward[a], Ana Alfirevic[a], B. Kevin Park[a] and Munir Pirmohamed[a]

Clinical phenotype

Causal chemical

Incidence before and after testing for HLA-B*5701

Country	Pre testing	Post testing	Reference
Australia	7%	<1%	Rauch et al, 2006
France	12%	0%	Zucman et al, 2007
UK (London)	7.8%	2%	Waters et al, 2007

HLA associations

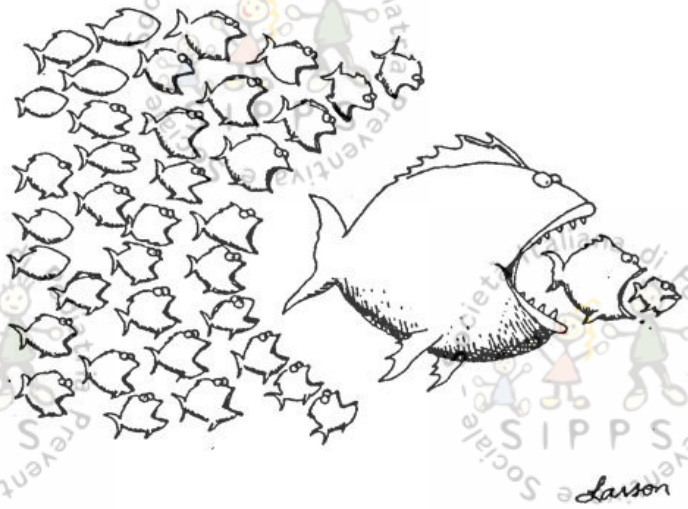
Drug	HLA association
Flucloxacillin	HLA-B*5701
Co-amoxiclav	HLA-DRB1*1501-DQB1*0602
Lumiracoxib	HLA-DRB1*1501-DQB1*0602
Ximelagatran	HLA-DRB1*07-DQA1*02
Lapatinib	HLA-DQA1*0201
Abacavir	HLA-B*5701
Carbamazepine	HLA-B*1502

Fig.3.15 – Focomelia da talidomide (da Toxipedia).

The New Science philosophy

Facilitates growth of knowledges ….

Top-bottom & bottom-up



multidisciplinarity & complementarity

Space for reflection and imagination !

Patient Profiling: RACE
a new methodology: fourth dimensional risk profile

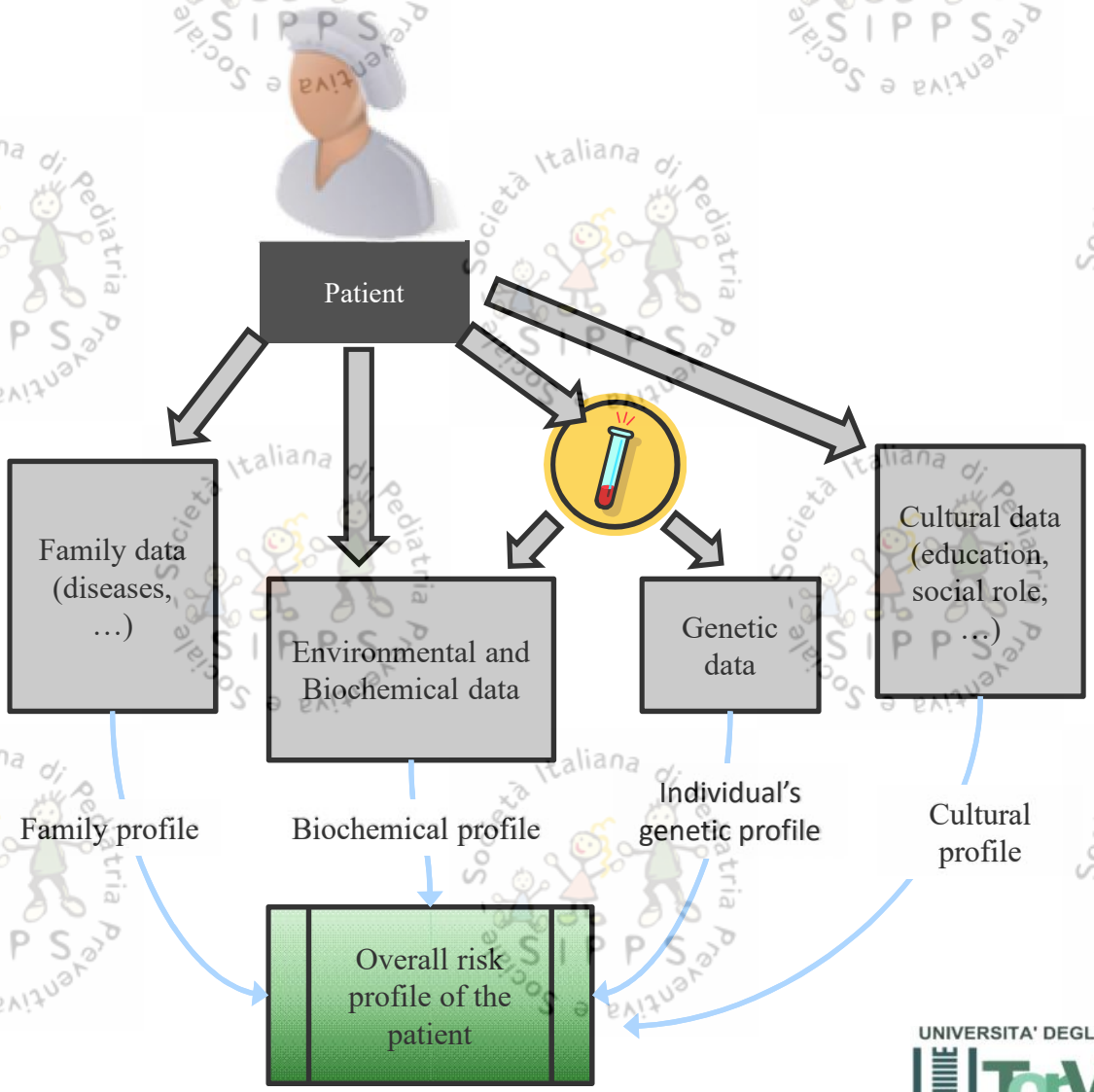


Figure 1. Comparison between clinical, genetic and integrated risk assessment.

Romeo, Novelli, Ferrari, Talamo, 2016

Drugs approved for neuromuscular disorders: beyond replacement therapy

MPS I: Aldurazyme®, 27 July 2009

MPS II: Elaprase, 08 January 2007

Infantile-onset Pompe Disease: Lumizyme 1st Aug 2014

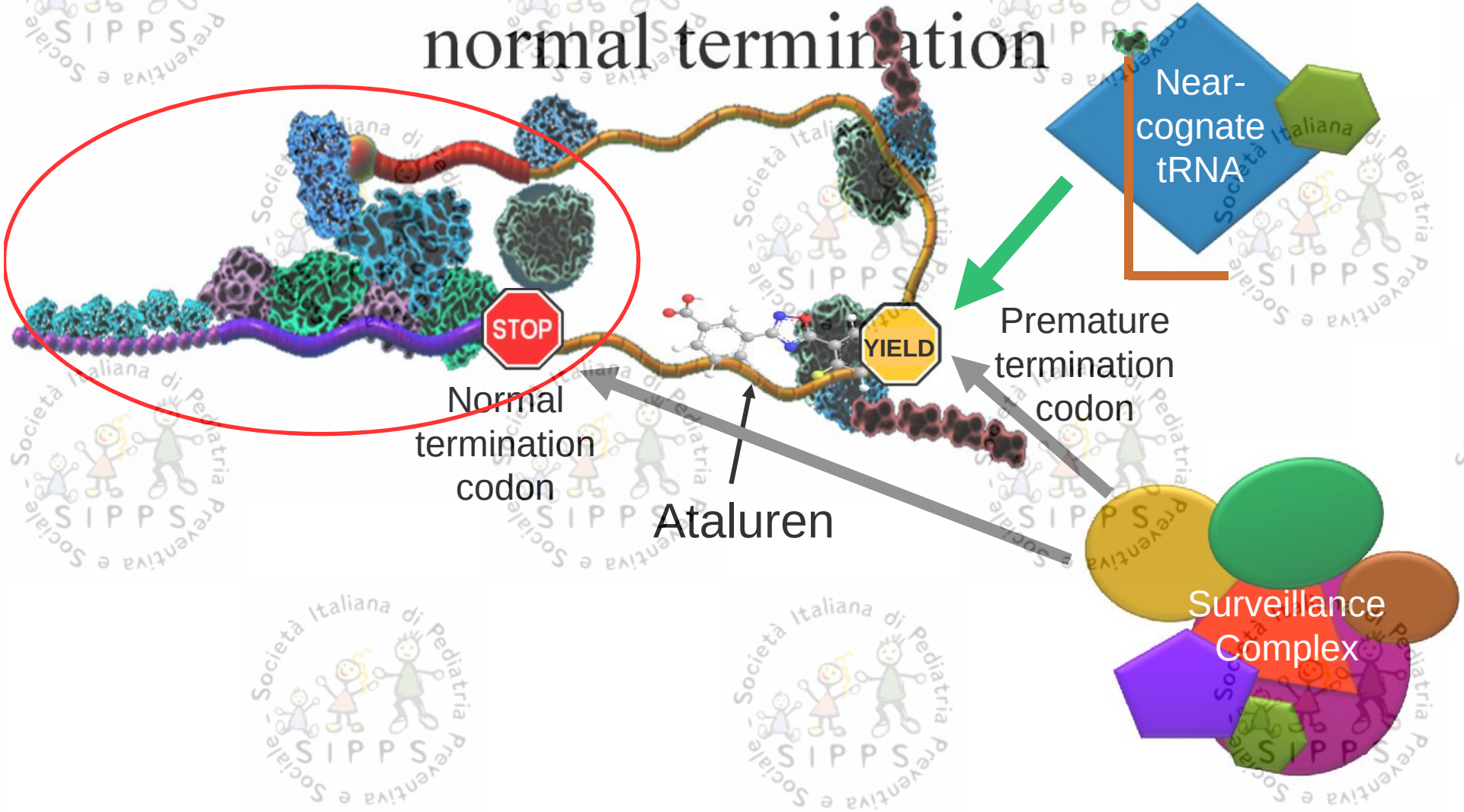
Morquio A Syndrome - MPS Iva: Vimizim, 28th April 2014

nm Duchenne MD: Translarna, 31st July 2014

Lipoprotein lipase deficiency: Glybera, 2014

Strimvelis, ADA-SCID , June 2016

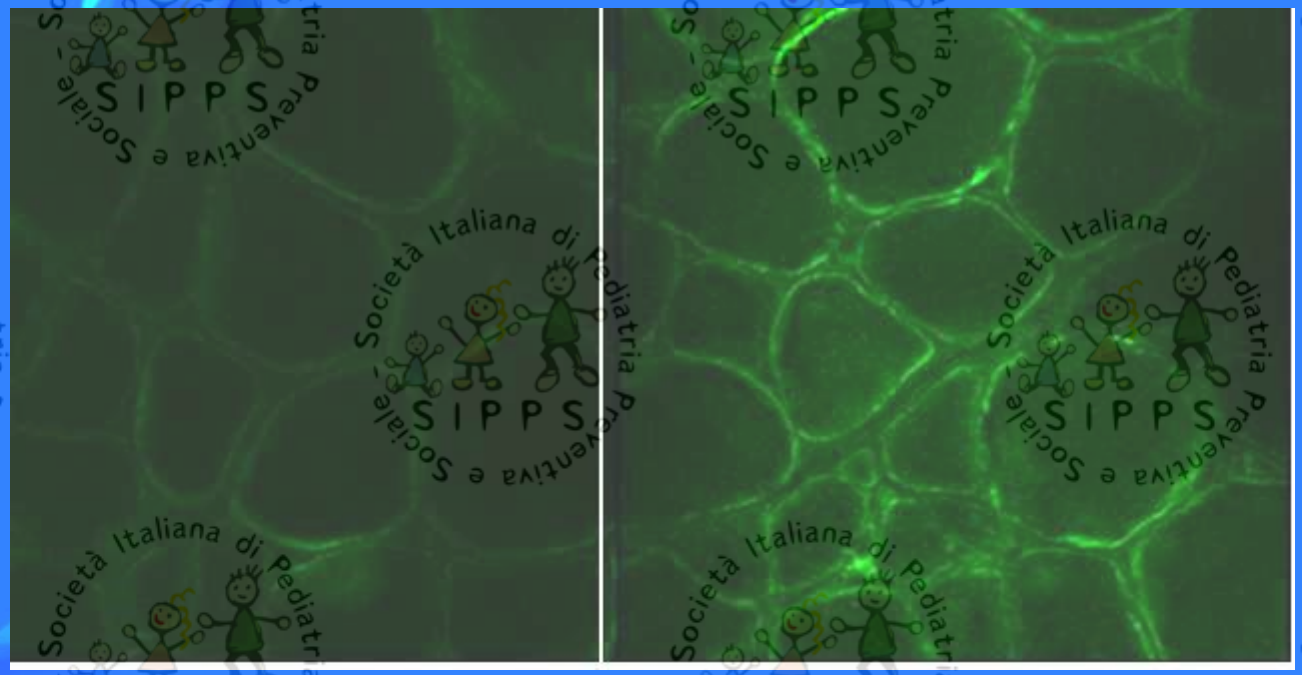
Premature termination differs from normal termination



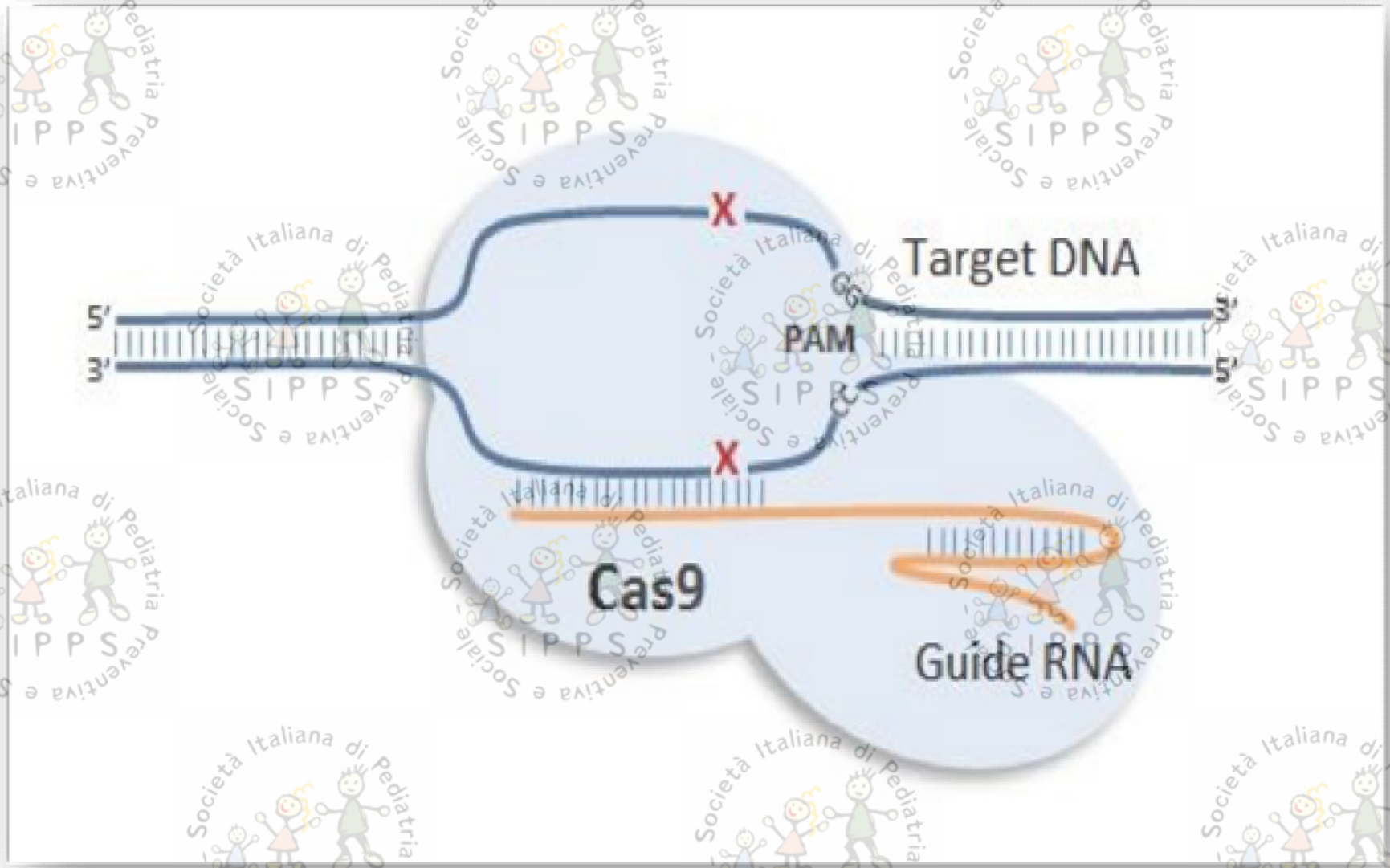
OPEN ACCESS Freely available online PLOS | ONE

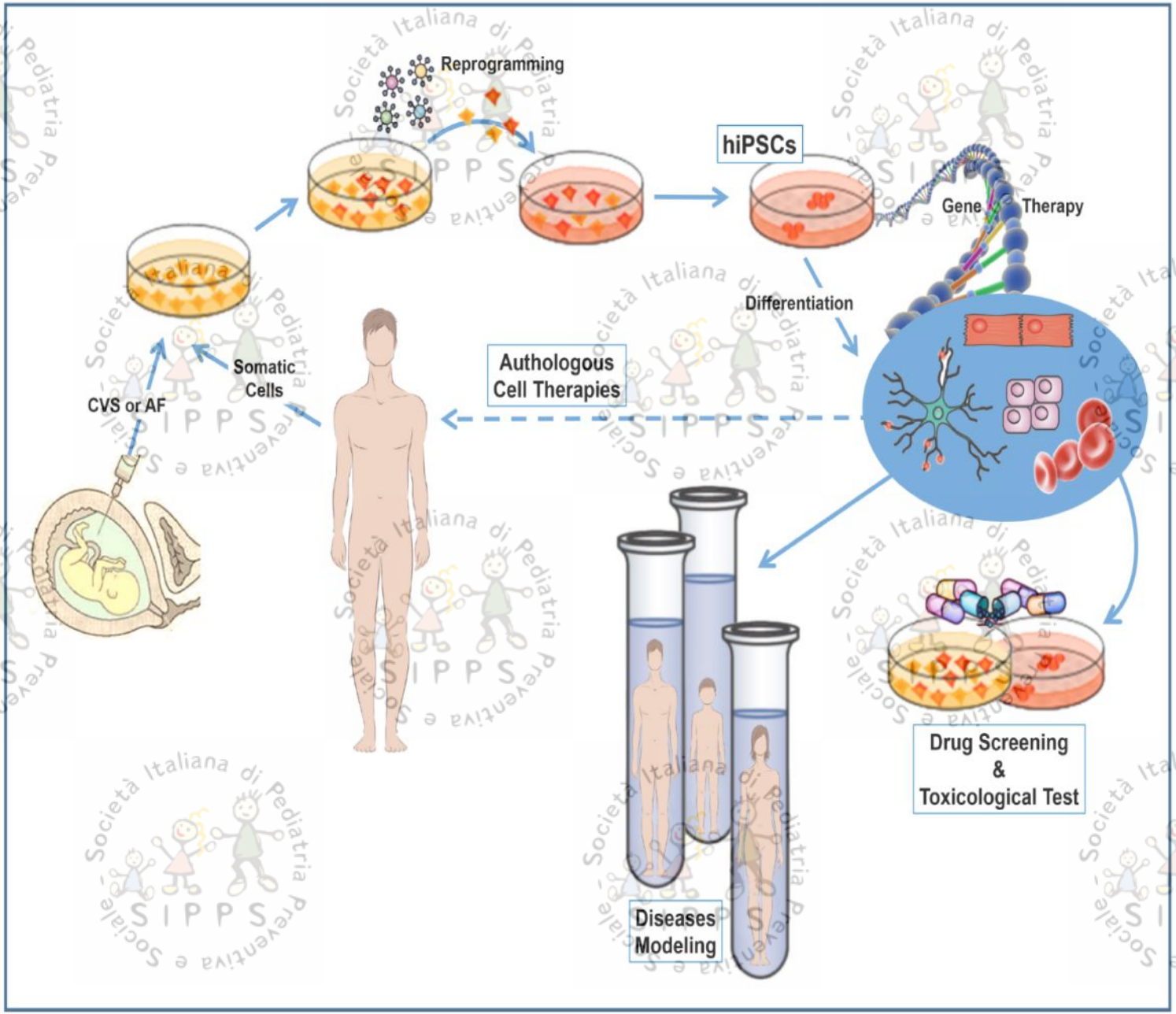
Phase 2a Study of Ataluren-Mediated Dystrophin Production in Patients with Nonsense Mutation Duchenne Muscular Dystrophy

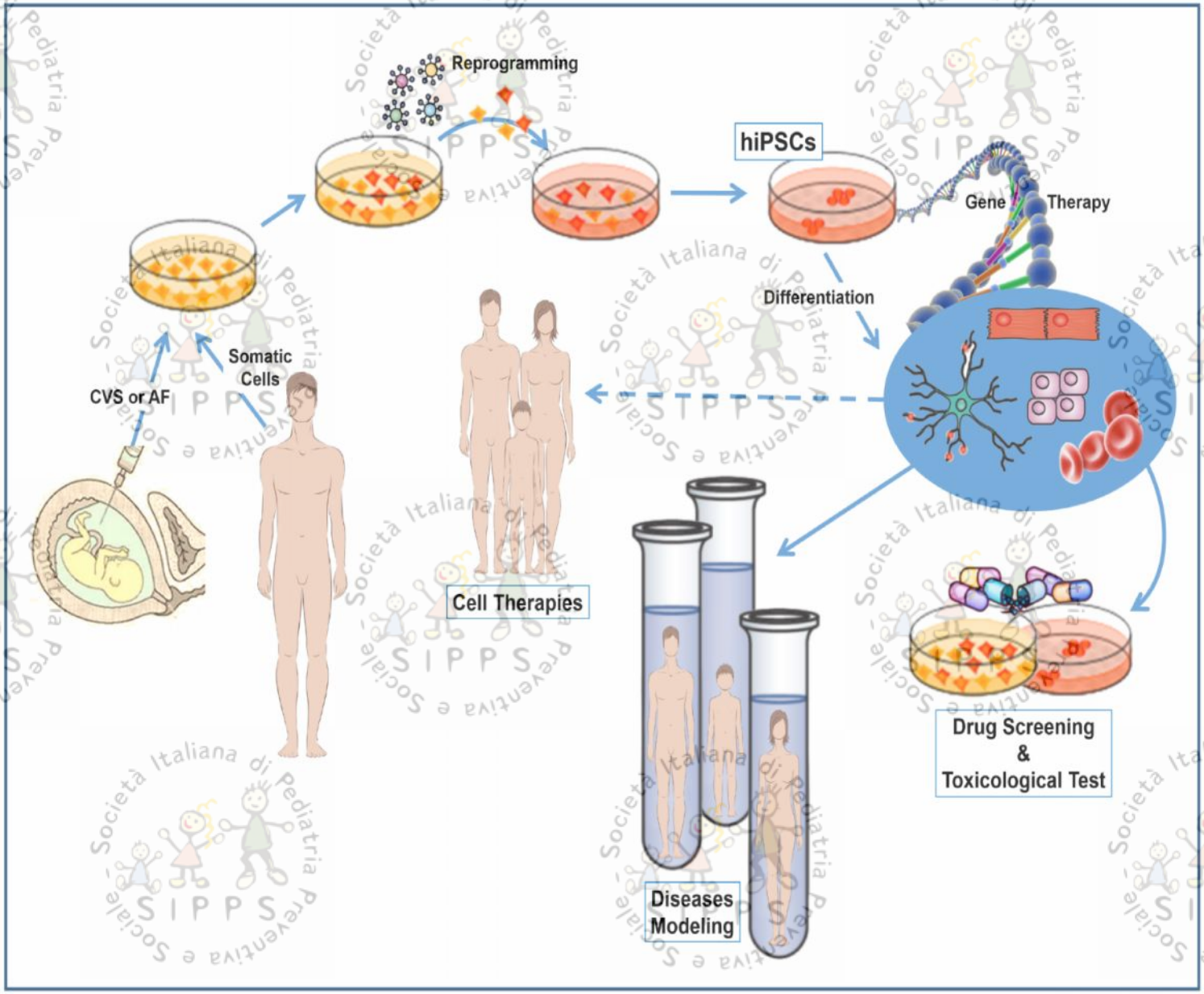
Richard S. Finkel[1,2*], Kevin M. Flanigan[3¤a], Brenda Wong[4], Carsten Bönnemann[1¤c], Jacinda Sampson[3¤b], H. Lee Sweeney[5], Allen Reha[6], Valerie J. Northcutt[6], Gary Elfring[6], Jay Barth[6], Stuart W. Peltz[6]



61% of patients showed an increase in dystrophin staining in 28 days of ataluren treatment


Target DNA
5′
3′
PAM
3′
5′
Cas9
Guide RNA


Reprogramming
hiPSCs
Gene
Therapy
Differentiation
Somatic
Cells
CVS or AF
Authologous
Cell Therapies
Drug Screening
&
Toxicological Test
Diseases
Modeling


Reprogramming
hiPSCs
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Drug Screening
&
Toxicological Test

.. If only we could read the language, the DNA of tuna and starfish would have the word "sea" written in the text…

..

Richard Dawkins