Genetic Lab Insights: Methodologies, Prevention and Diagnostic Advances

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Outline

1. GENETICS:
    1. PREVENTION
    2. DIAGNOSTIC
    3. PERSONALIZED MEDICINE
2. ANIMAL MODELS IN GENETICS
3. RESEARCH

Há mais de

6.000

doenças RARAS

conhecidas

80% das

DOENÇAS **RARAS**

são

GENÉTICAS

70% das

Doenças

Genéticas Raras

surge na INFÂNCIA

Há **300**milhões

de pessoas

que vivem com

uma Doença RARA

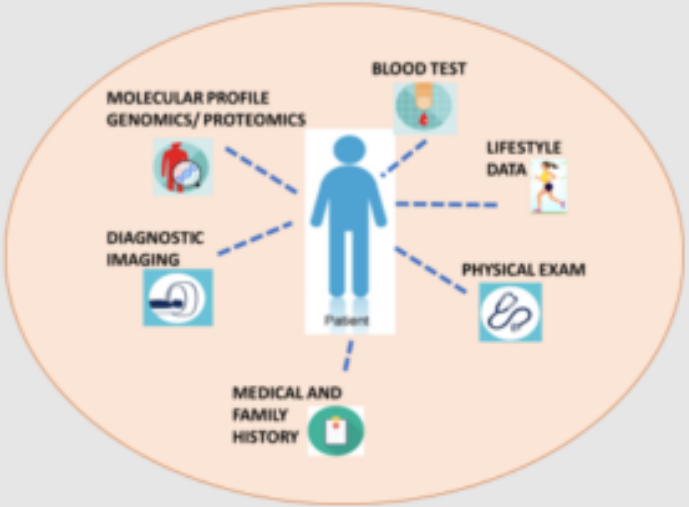
em todo o MUNDO

Same laboratory: Differents approachs

PREVENTION

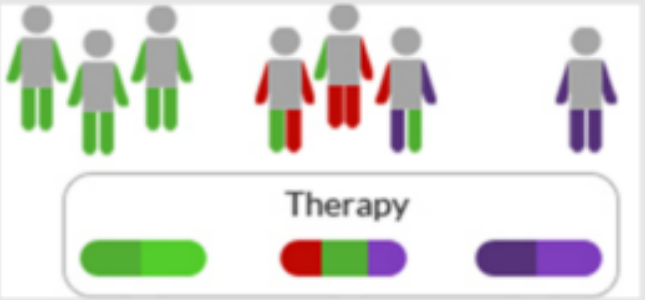
Early detection of patients at risk, Improve preventive measures (individual/collective)

DIAGNOSIS



Accurate disease diagnosis enabling individualized treatment strategy

TREATMENT



Improved outcomes through targeted treatments and reduced side effects

Inclusion Criteria: Wilson and Jungner Foundations

Early Impact Assessment

Conditions must demonstrate early onset with significant health consequences requiring immediate intervention.

Treatment Availability

Effective, timely therapeutic interventions must be accessible and proven to improve patient outcomes.

Screening Reliability

Robust, cost-effective screening methodologies with high sensitivity and specificity are essential.

- The condition sought should be an important health problem.
- There should be an accepted treatment for patients with recognized disease.
- Facilities for diagnosis and treatment should be available.
- There should be a recognizable latent or early symptomatic stage.
- There should be a suitable test or examination.
- The test should be acceptable to the population.
- The natural history of the condition, including development from latent to declared disease, should be adequately understood.
- There should be an agreed policy on whom to treat as patients.
- The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
- Case-finding should be a continuing process and not a “once and for all” project.

Phenylketonuria

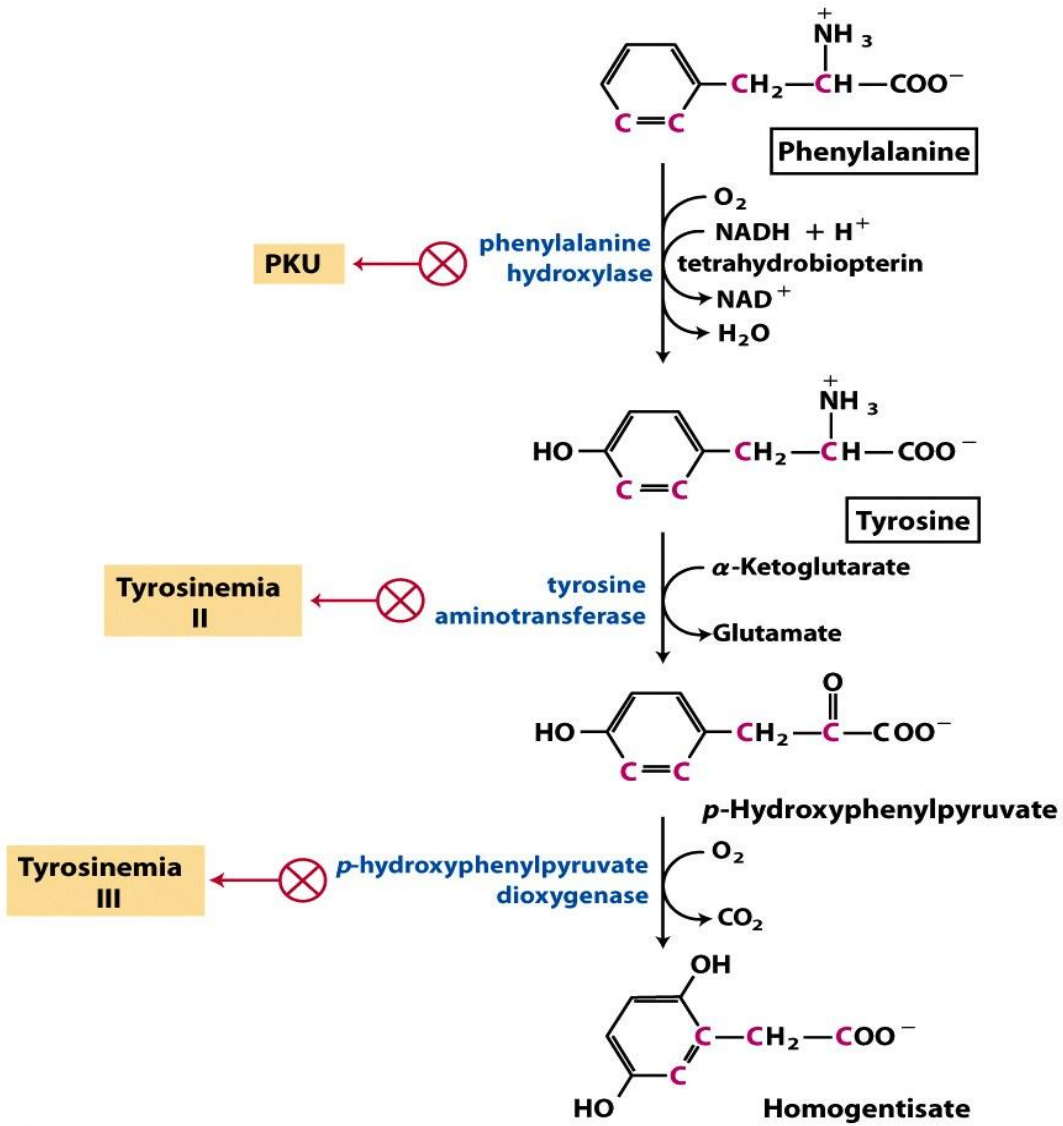


Figure 18-23 part 1
Lehninger Principles of Biochemistry, Fifth Edition

Molecular Genetics: From Confirmation to First-Line

Traditional Paradigm

Molecular genetic testing historically served as a confirmatory tool following clinical and biochemical characterization. Identification of pathogenic variants confirmed suspected diagnoses and enabled cascade testing of family members.

Contemporary Approach

Short-read next-generation sequencing technologies enable simultaneous analysis of multiple genes through panels, exomes, or genomes. This approach has transformed molecular genetics from confirmatory to first-line diagnostic testing.

Massive Variation in Gene Selection in NBSeq – pilot studies

4,390	134-4,299	1,85 – 9,43%	93%
Total Unique Genes	**Gene List Range**	**Screening positive rate**	**Low Agreement**
Genes included across at least one of the 27 NBSeq programs analyzed	Number of genes included per program, with a median of 306 genes	Results of nine programs with published screening results (68,628) infants,	Of genes were included by 10 or fewer programs, showing substantial heterogeneity

The analysis revealed striking discordance across NBSeq programs. Most genes had minimal consensus: 4,089 genes (93%) were included by 10 or fewer programs, and 3,793 genes (87%) appeared on five or fewer lists. This heterogeneity reflects differences in international disease prevalence, healthcare system capacity, treatment availability, and individual program objectives.

Minten T, *et al*. Genet Med. 2025 Jul;27(7):101443.

Gene Panel Design Considerations

Variant Interpretation Challenges

60+

LSDs Identified

1000+

Gene Variants

40%

Uncertain Significance

Variant interpretation remains the biggest challenge as more genotypes are generated. Significant advances through experience sharing, population databases, and functional studies are improving LSD gene-specific interpretation guidance.

Gene Panel Design Considerations – Pilot studies

Gene Selection

Panels should include only genes with validated disease associations for lysosomal storage disorders, guided by ClinGen clinical validity assessments and expert consensus.

Variant Detection

Comprehensive analysis must address technical challenges including pseudogene interference (GBA1, NEU1, IDS), large deletions, and inversions. Supplemental testing often required.

Allelic Spectrum

Understanding common pathogenic variants (deletions for GALC, HEXB, CLN3, CTNS; inversions for IDS) ensures appropriate assay design and interpretation frameworks.

NBSeq Challenges

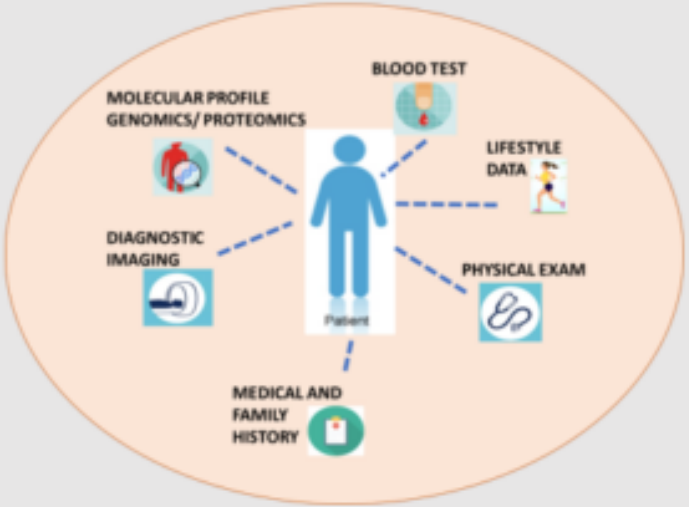
1. Technical Feasibility	• Response Time • Result Confirmation • Result Interpretation
2. Economic Considerations	• Sequencing Price • Specialized Human Resources • Computing Resources • Confirmatory Tests
3. Medical Considerations	• Gene Selection Criteria • Interpretation of Results • How to Report
4. Legal, Ethical and Psychological Implications	• Need for Informed Consent • Child welfare • Benefits for siblings and parents • Late-onset diseases

Same laboratory: Differents approachs

PREVENTION

Early detection of patients at risk, Improve preventive measures (individual/collective)

DIAGNOSIS



Accurate disease diagnosis enabling individualized treatment strategy

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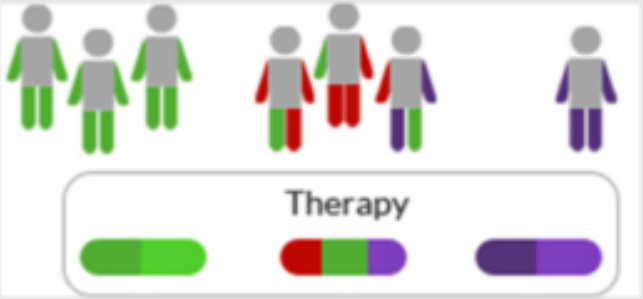
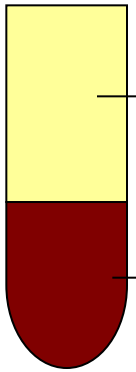


Improved outcomes through targeted treatments and reduced side effects

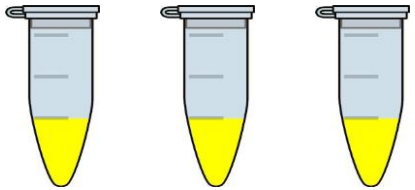
Centrifugação



Plasma

Centrifugação

Plasma



Aliquotas (-20º C)

sedimento com
Leucócitos e
Eritrócitos

Misturar com Solução de Lise

Armazenar -20º C

DNA

Understanding Inheritance: The Four Classic Patterns

Autosomal Dominant

One mutated gene copy causes disease. Affected individuals have 50% chance of passing condition to offspring.

Example: Huntington's disease

Autosomal Recessive

Two mutated copies needed for disease expression. Carriers remain unaffected but can transmit mutations.

Example: Cystic Fibrosis

X-linked Inheritance

Mutations on X chromosome show distinct patterns. Males more severely affected due to single X chromosome.

Example: Haemophilia A

Mitochondrial Inheritance

Passed exclusively from mother to all children. Affects cellular energy production in both sexes.

Example: Leber's optic neuropathy

Next Generation Sequencing

Painéis Dirigidos

Análise focada em genes específicos associados a fenótipos clínicos definidos

Exoma

Sequenciação de todas as regiões codificantes do genoma humano

Genoma Completo

Análise abrangente incluindo regiões não codificantes e estruturais

Bioinformatic tools

https://www.ensembl.org/index.html/

LOVD - An Open Source DNA variation database system

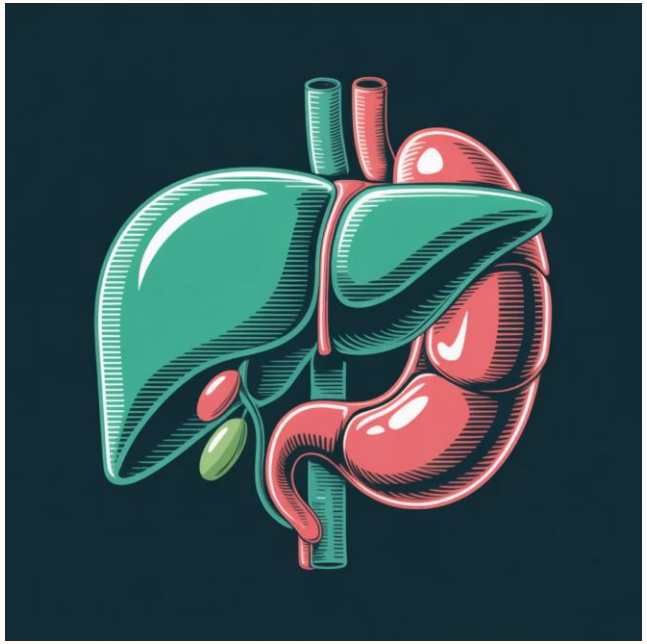
https://varchat.engenome.com/

https://franklin.genoox.com/clinical-db/home/

http://v1.marrvel.org/search/gene/grn/

What is Gaucher Disease?

- A rare inherited lysosomal storage disorder caused by mutations in the *GBA1* gene
- Deficiency of **glucocerebrosidase (GCase)** enzyme prevents breakdown of fatty substances
- Glucosylceramide\Glucosylsphingosine accumulates in organs: spleen, liver, bone marrow, causing progressive damage



1	2	3
Type 1 (Non-neuronopathic) neuronopathic)	**Type 2 (Acute neuronopathic) neuronopathic)**	**Type 3 (Chronic neuronopathic) neuronopathic)**
Most common form	**Severe, early onset** in infancy	**Milder neurological symptoms**
Affects spleen, liver, bones	Rapid neurological decline	Onset in childhood
No brain involvement	Usually fatal by age 4	Progressive brain involvement
Variable severity and age of onset	Most aggressive form	Intermediate severity

Fabry Disease: A Rare Genetic Disorder

X-linked Inheritance

Lysosomal storage disorder caused by α-galactosidase A deficiency affecting predominantly males

Cellular Accumulation

Progressive accumulation of glycosphingolipids, primarily globotriaosylceramide (Gb3) in lysosomes

Multi-organ Impact

Systemic involvement affecting kidneys, heart, nervous system, skin, and gastrointestinal tract

Clinical Variability

Manifestations vary between classic and late-onset forms, with gender differences

Diagnostic Markers and Tools

01

Clinical Suspicion

Recognition of characteristic symptoms, family history, and multi-organ involvement triggers diagnostic workup.

02

Enzyme Activity Assays

Gaucher: Blood test measuring glucocerebrosidase activity in leucocytes or dried blood spots.

Fabry: Alpha-galactosidase A activity assay—reliable in males, less so in female carriers.

03

Genetic Testing

Confirmatory DNA sequencing identifying specific *GBA* or *GLA* gene mutations, essential for definitive diagnosis and family screening.

04

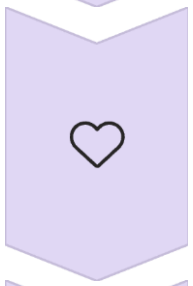
Biomarker Monitoring

Elevated **glucosylsphingosine** (lyso-Gb1) in Gaucher disease and **globotriaosylsphingosine** (lyso-Gb3) in Fabry disease aid diagnosis and treatment monitoring.

Diagnostic Utility Across Fabry Phenotypes

Classic Males

Markedly elevated Lyso-Gb3 correlates directly with severe multi-organ disease manifestations and early symptom onset



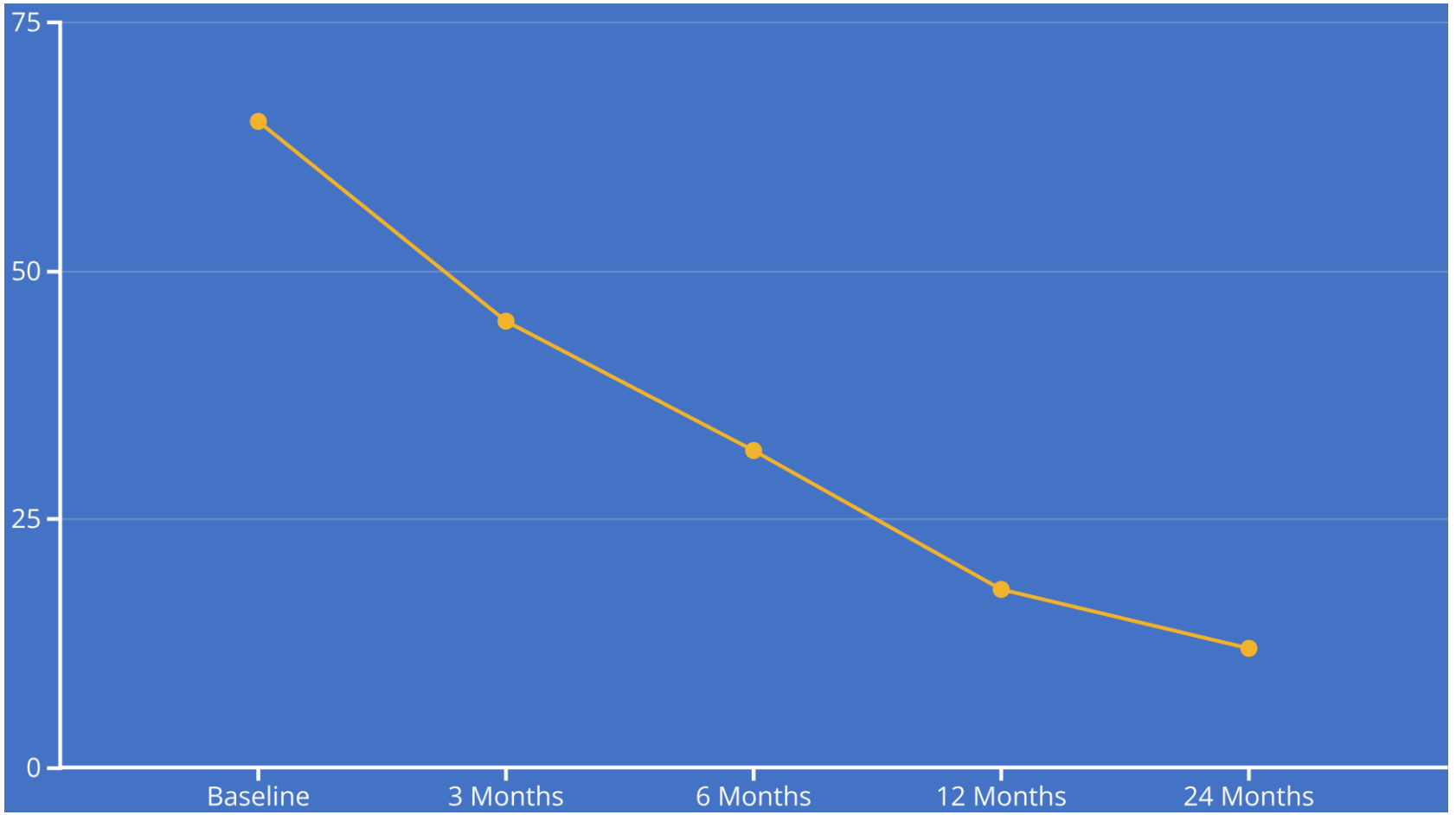
Some Late-Onset Cardiac forms

Intermediate Lyso-Gb3 levels effectively distinguish distinguish patients from healthy controls,

Female Heterozygotes

Variable Lyso-Gb3 levels reflect the broad phenotypic phenotypic spectrum, serving as valuable adjunct to to genetic testing

Treatment Evolution: LysoGb3 Level Changes



Typical response pattern showing progressive reduction in LysoGb3 levels following enzyme replacement therapy initiation.

Hunter disease: IDS Gene Mutation

01

Mucopolyssacharidosis, type II

Hunter disease, scientifically known as Mucopolysaccharidosis type II (MPS II), is a rare genetic disorder that predominantly affects males. This condition arises from a deficiency of the enzyme iduronate 2-sulfatase (I2S), .

02

X-Linked Recessive Inheritance

Hunter disease follows an X-linked recessive pattern, meaning the defective gene is located on the X chromosome.

03

IDS Gene Mutations

Mutations in the IDS gene significantly reduce or completely eliminate the production of functional I2S enzyme.

04

Cellular Accumulation

Without adequate I2S enzyme, glycosaminoglycans build up inside lysosomes, progressively damaging tissues and organs.

05

Inheritance Risk

Carrier mothers have a 50% chance of passing the mutation to each child—sons become affected whilst daughters become carriers.

Lyssomal Storage disorders - The Challenge of Early Detection

Patients with LSDs are primarily identified after presenting with symptoms following a diagnostic odyssey

Earlier diagnosis through family history or newborn screening enables access to beneficial treatments

Treatment Landscape

Enzyme Replacement

Available for multiple disorders including Gaucher, Fabry, and MPS types

Substrate Reduction

Reduces accumulation of harmful substances in affected cells

Transplantation

Bone marrow or organ transplant for severe cases when initiated early

Gene Therapy

FDA-approved for metachromatic leukodystrophy, trials ongoing for others

Same laboratory: Differents approachs

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Early detection of patients at risk, Improve preventive measures (individual/collective)

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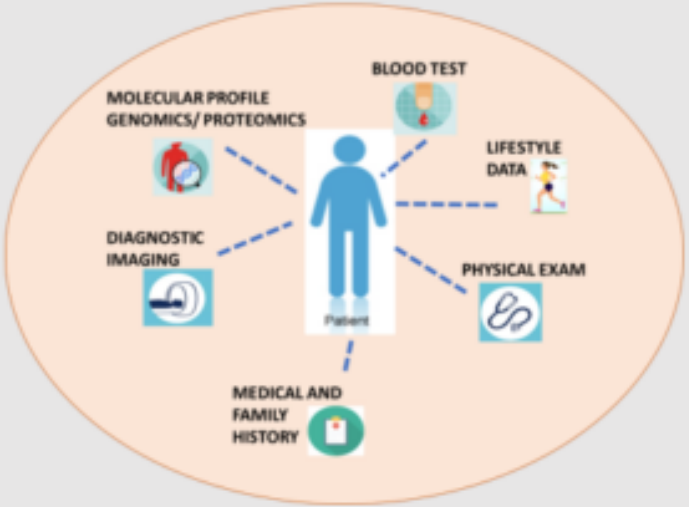

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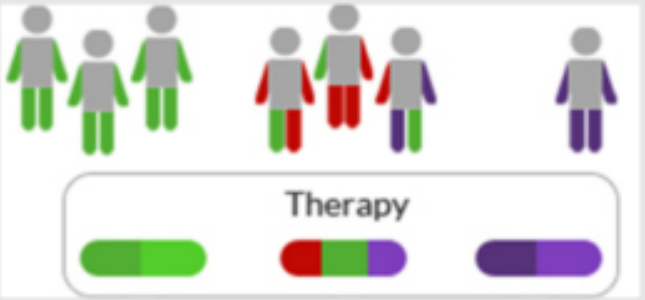

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Personalized medicine

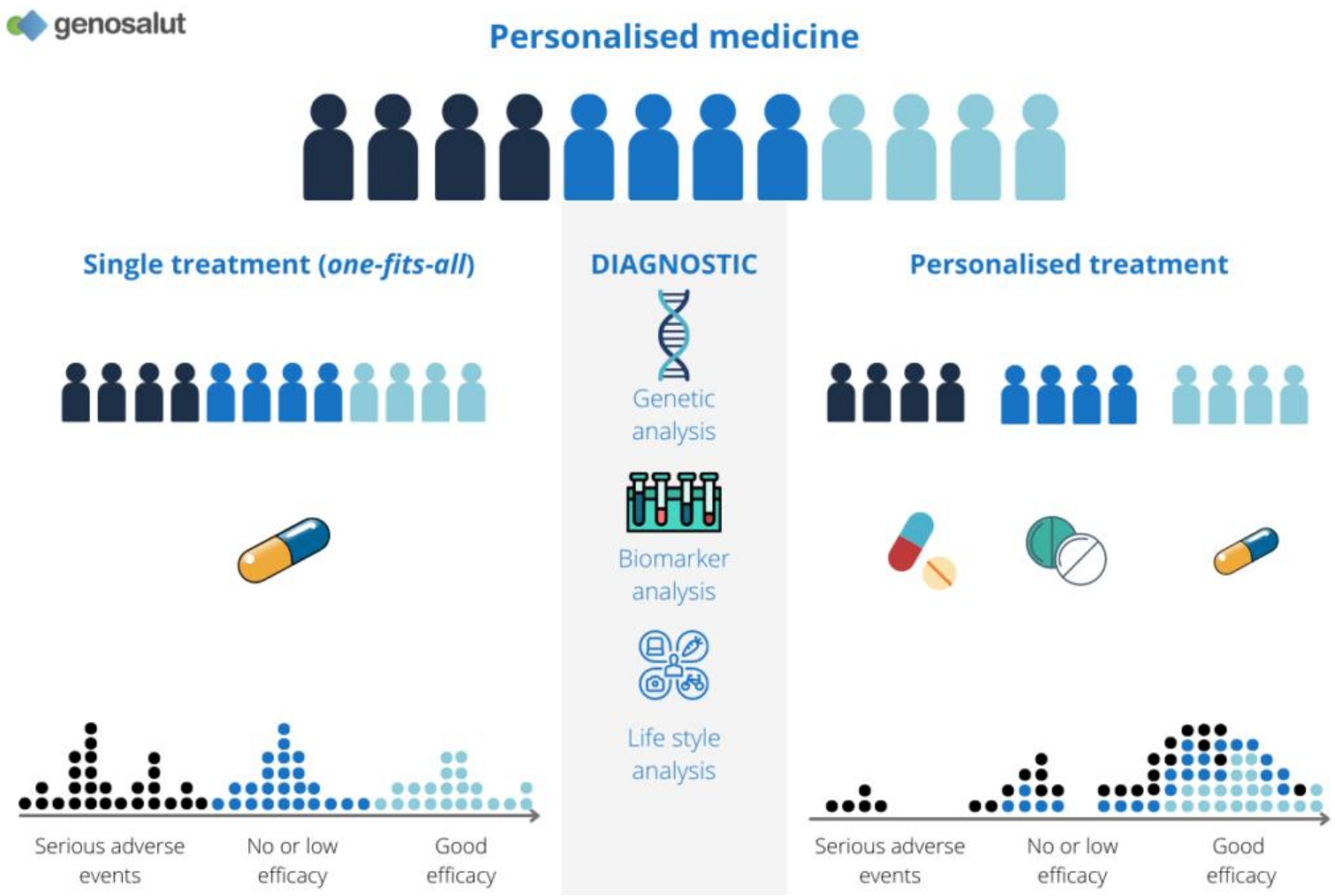


Image from https://www.genosalut.com/en/news/healthy-lifestyle/what-is-personalised-medicine/

PM and Oncology

Cancer:

5-fluorouracil (5-FU), capecitabine, and tegafur - colorectal, breast, head and neck, pancreatic, and gastric cancers.

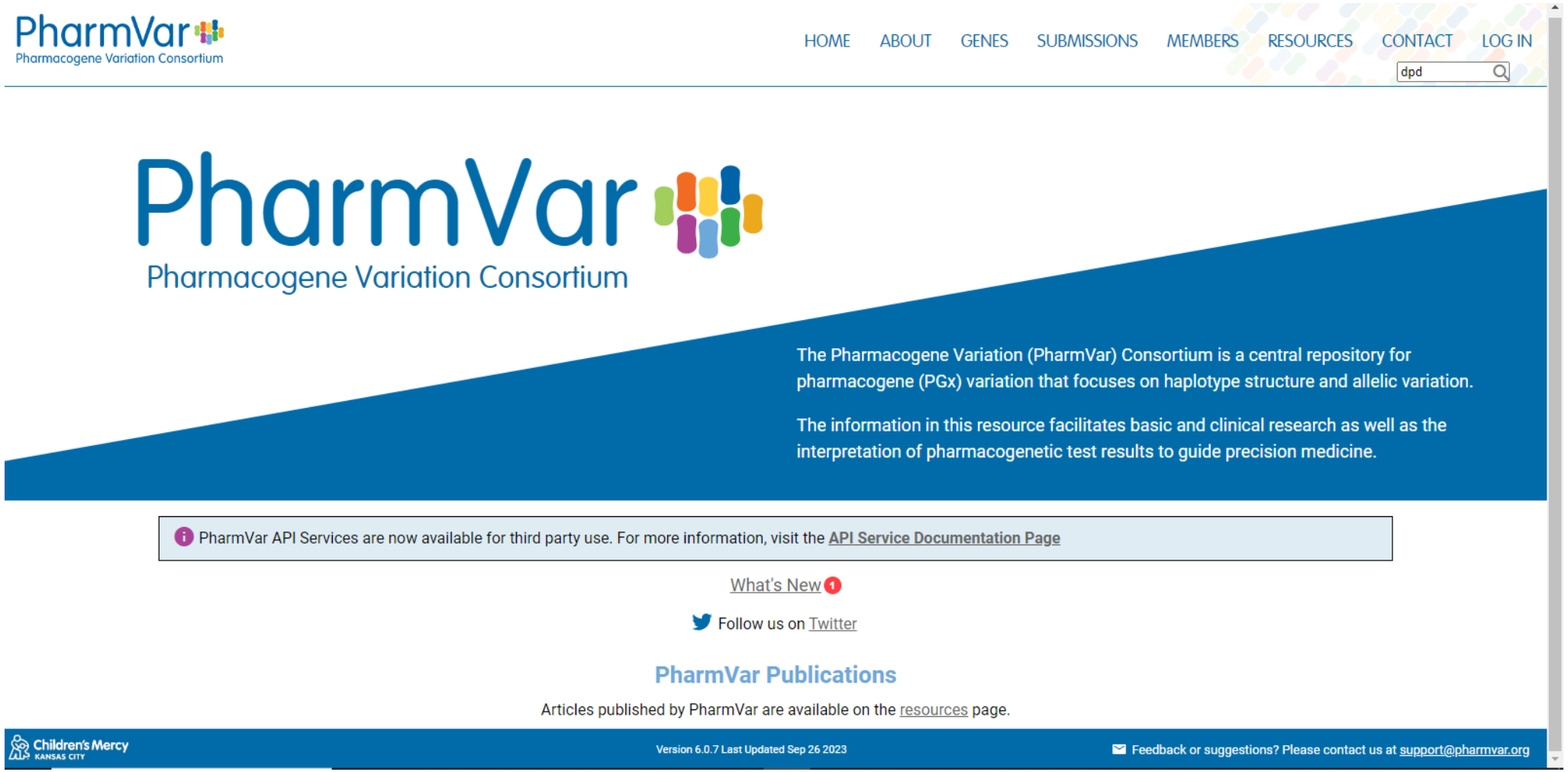
Treatment

Fluoropyrimidines - Although crucial for the tratment, patients may experience severe toxicity.

The dihydropyrimidine dehydrogenase (DPD) enzyme is the first and rate-limiting enzyme in the catabolic pathway. If DPD activity is lower, may increase a person's risk of toxicity.

https://www.pharmvar.org/

PM and Oncology



PM and Lysosomal Storage disorders
Gaucher

- Gaucher Deficiency in *GBA* gene leading to a deficiency in B-glucosidase.
- **Main treatment Options -** Enzyme replacement therapy (ERT) and Substrate reducing therapy (SRT)
- Cerdelga(Eliglustat) – belongs to SRT class, long-term treatment of adult patients with Gaucher disease type 1
- Eliglustat is metabolized by Cytochrome P450 2D6 (CYP2D6), expressed essentially in the liver.
- Potential toxicity might be observed in patients concurrently treated with known CYP2D6 inhibitors or which are power metabolizers.
- https://www.pharmvar.org/gene/CYP2D6

PM and Lysosomal Storage disorders
Gaucher

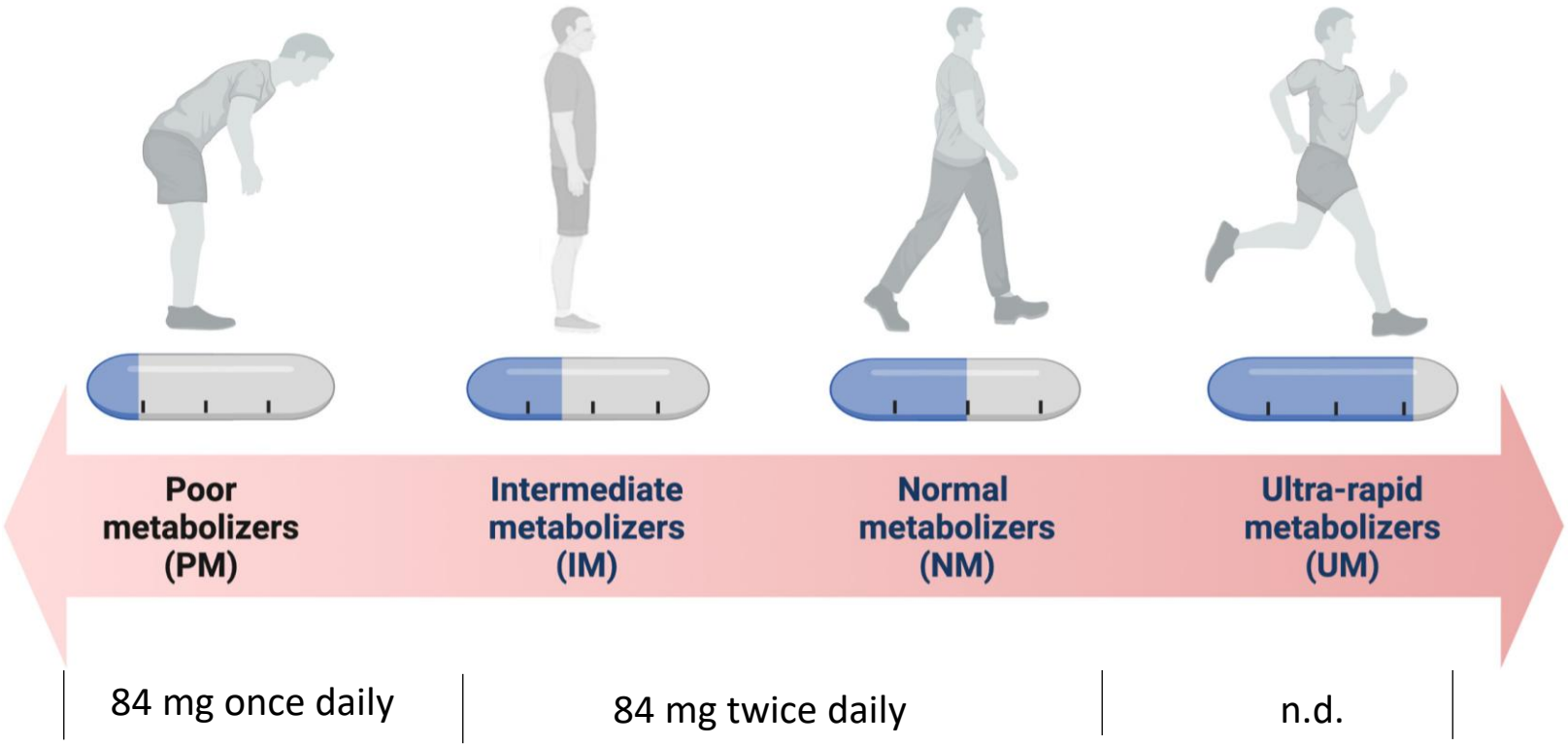
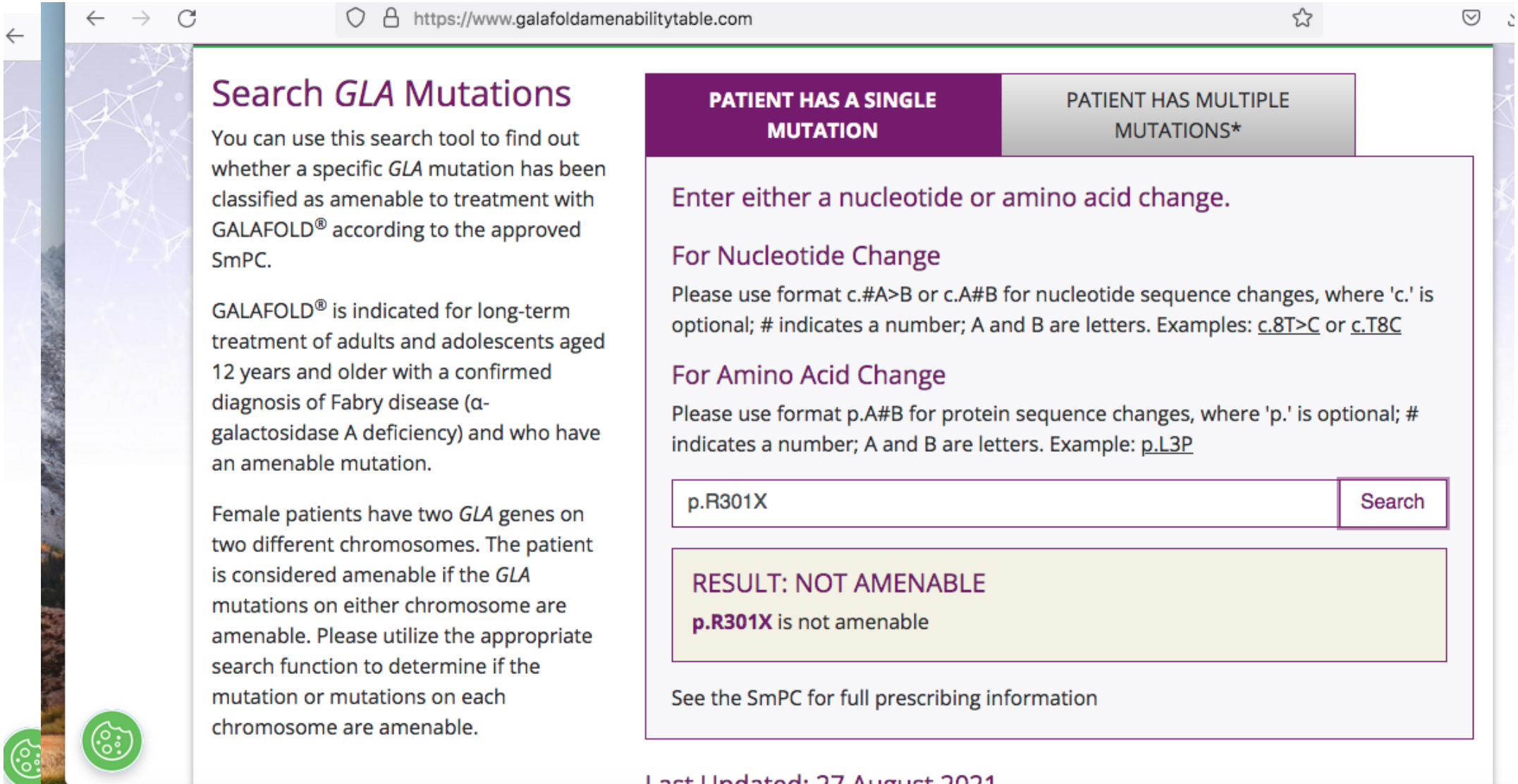


Image from Biomed Pharmacother. 2021 Oct;142:112060.

PM and Lysosomal Storage disorders
Fabry



Animal Models for Studying Genetic Disorders: Unlocking Human Disease Mysteries

Exploring how model organisms help us understand, diagnose, and treat inherited human diseases

Shared Genetic Heritage

Many animals share a remarkable percentage of key genes and biological pathways with humans, creating natural parallels for study.

Translational Power

This genetic conservation enables researchers to extrapolate findings from animal studies directly to human physiology and disease mechanisms.

Accelerated Discovery

Understanding disease in animals fast-tracks therapeutic development for human patients with genetic disorders.

Small but Mighty: The Usual Suspects

Mice (Mus musculus)

Share 95% genome similarity with humans, high reproduction rates, and low maintenance costs make mice the workhorse of genetic research.

Fruit Flies (Drosophila)

Pioneered early gene mapping techniques and mutant screening approaches that revolutionized genetics.

Roundworms (C. elegans)

Transparent bodies reveal nervous system development in real-time, ideal for aging and neural studies.

Zebrafish (Danio rerio)

Transparent embryos enable direct observation of organ development and rapid gene function identification.

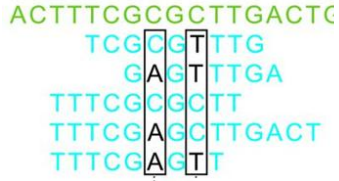
A MODERN LAB

- Classical biochemical and molecular analyses negative or unclear

- No sample for biochemical analysis

- Mutations in several genes could explain the phenotypez

NGS-TARGETED GENES PANELS
Clinical Exome
Whole Exome Sequencing
RNA sequencing

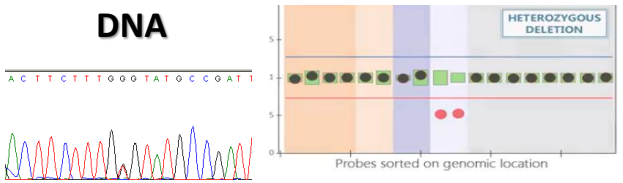


Data Analysis Pipeline

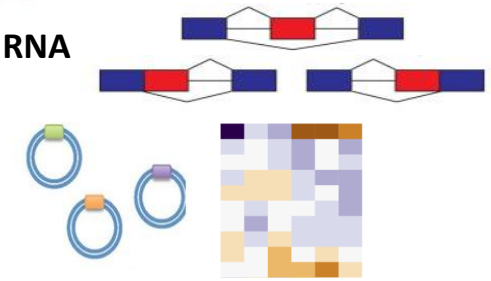
in silico prediction of mutation effect on gene function

Experimental Studies

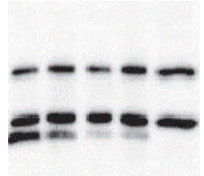
DNA

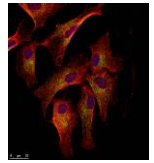


RNA



Protein/Biomarkers





Do you want to know your future?