



# Fabry masterclass

Scientific Coordinator  
**Prof. Olga Azevedo**

## Newborn screening: challenges and risks



🌐 **Network**  
Hereditary Metabolic  
Disorders (MetabERN)

● **Member**  
Hospital Senhora da  
Oliveira, Guimarães,  
FPF — Portugal

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# Disclosures

The speaker declares no conflicts of interest

# Newborn screening – the beginning

## Early 1960s:

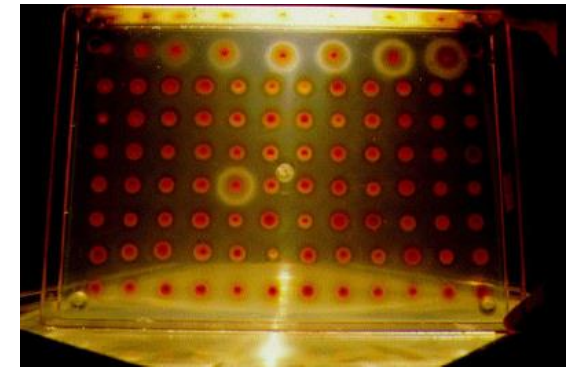
Robert Guthrie started a neonatal screening for Phenylketonuria (PKU) - based on a bacterial inhibition test to detect high levels of phenylalanine, in DBS collected in the first days of life.

New diseases started to be screened in different populations



need for guidelines

**Wilson and Jungner principles**  
Wilson J and Jungner G, WHO, 1968



# Wilson & Jungner principles

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. 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.
10. Case-finding should be a continuing process and not a “once and for all” project.

Wilson JMG, Jungner G (1968) Principles and practice of screening for disease. Geneva: WHO.  
Available from: <http://www.who.int/bulletin/volumes/86/4/07-050112BP.pdf>

# “Expanded” newborn screening

## Early 1990s:

Tandem mass spectrometry (ms/ms) technology adapted to newborn screening



Rapid and simultaneous detection of more than 40 IEM ( amino acids, fatty acids, and organic acids metabolism), through the simultaneous analysis of amino acids and acylcarnitine profiles.



# Ms/ms screening for IEM

*Tandem mass spectrometry (ms/ms)*

1 sample → 1 analysis → 1 disease

	Phe	PKU
	TSH	CH

Cost-effective detection of very rare diseases



Re-tinking Wilson and Jungner principles

1 sample → 1 analysis → multiple diseases

	1 aa + acyl profile	> 40 IEM
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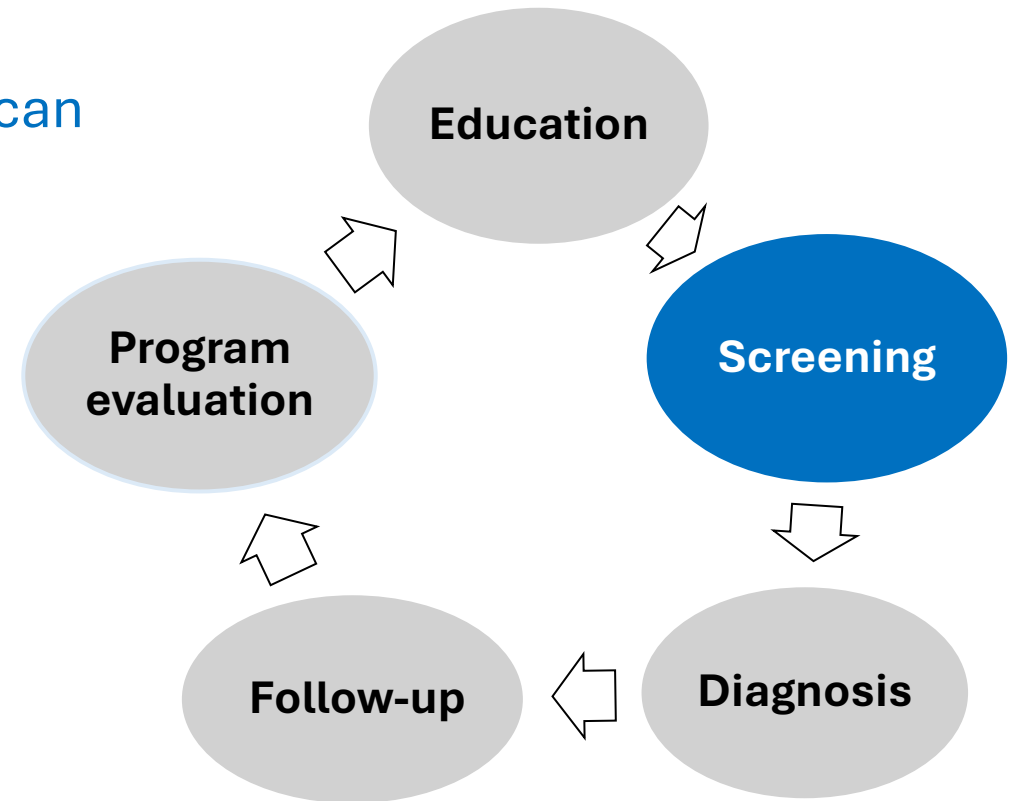
# Newborn screening - today



Organized complex public health prevention programs, aimed at the identification of newborns at risk for the development of an increasing number of serious conditions, for which early therapeutic intervention can prevent premature mortality or morbidity.

## Driven forces for the development of NBS programs:

- Development of new technologies, preferably multiplex
- Development of new NBS-suitable biomarkers
- Development of new and more cost-effective therapies



# Heterogeneity of NBS programs

- National or regional? Universal or dedicated?
- Mandatory or optional?
- Samples collection time (24h? 48h? 72h?)
- How many diseases screened? Which diseases?
- Which are the screening methods and algorithms?
- How are the results reported and to whom?
- Is therapy and follow-up assured by a specialized multidisciplinary team, for all positive cases?

**Difficult harmonization due to different economic, social and ethnic factors, typical of each population.**

# Heterogeneity of NBS programs

**Since 2006, USA** have a Recommended Uniform Screening Panel (RUSP), kept updated by an NBS expert group, responding to the U.S. Secretary of Health and Human Services :

37 recommended conditions

+

26 secondary conditions

Only 3 LSD recommended conditions: Pompe disease (2015), MPS I (2016) and MPS II (2022).

**Europe:** very large heterogeneity for NBS-programs, including the number of screened conditions ( 2 to > 20). No national screenings for LSD.

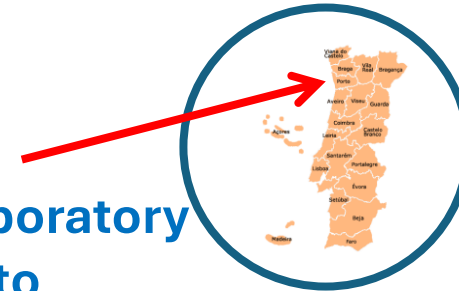
# Newborn Screening in Portugal

No-mandatory program covering the whole country and including all newborns born in Portugal.

>99% coverage  
10 days average referral age for positive cases.

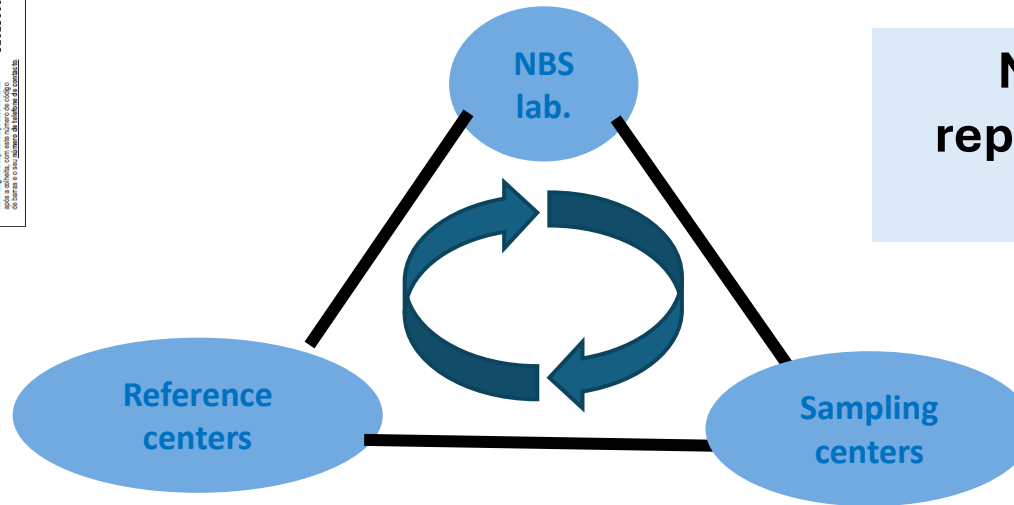
One National Laboratory  
INSA – Porto

350 samples/day  
85,000 samples/year



PROGRAMA NACIONAL DO RASTREIO NEONATAL	
Se esta colheita for uma repetição, assinale com uma cruz <input type="checkbox"/>	
Nome da Mãe _____	
Endereço _____	
Localidade _____ C. Postal _____	
Nascimento _____	Idade Gestacional _____
Colheita _____	N.º Uterino da Mãe (Obrigatório) _____
Alimentação - Peito <input type="checkbox"/> Outra <input type="checkbox"/>	Sexo <input type="checkbox"/> M <input type="checkbox"/> F <input type="checkbox"/>
Medicação - Qual? _____	Gêmeos <input type="checkbox"/> 1 <input type="checkbox"/> 2 <input type="checkbox"/> 3 <input type="checkbox"/>
Local de Colheita _____	Distrito _____
COLABORE CONNOSCO no pedinho do bebé pode estar o seu futuro	
ENVIAR PARA: INSTITUTO NACIONAL DE SAÚDE DOUTOR RICARDO JORGE Unidade de Rastreio Neonatal, Metabolismo e Genética Rua Alexandre Heróulano, 521 4000-055 Porto Telef. 223 401 168 / 76 / 770	

Card for sample collection, after 36h feeding



Normal results are reported on the internet (printable report)

# Newborn Screening in Portugal

PKU	CH	MS/MS (24 IEM)	CF	SCD	SMA	SCID (pilot study)
1979	1981	2004 - 2009	2013 - 2018	2021 - 2022	2022 - 2024	2025

Disease	Birth prevalence
IEM	1: 2,232
CH	1: 2,752
CF	1: 10,322
SCD	1: 2,136
SMA	1: 15,520
Total	1: 694

**Global results (1979 – 2024)**  
**4,309,181 screened newborns**  
**2,796 positive cases identified**

# Newborn Screening for LSD

2001 – 2004: [Chamoles and coworkers](#) develop DBS-based activity assays for several lysosomal enzymes using fluorogenic substrates (4MU based assays, including for alpha-Gal A).

2004: [Michael Gelb](#) reports the application of ms/ms to activity assays for lysosomal enzymes using enzyme-specific substrates and internal isotopically labeled standards.

High-throughput multiplex methodology (ongoing studies):

DMF (Digital Microfluidic Fluorometry) - [enzyme activities](#)

ms/ms – [enzyme activities](#)

LC-ms/ms – [enzyme activities and accumulated products](#)



Molecular analysis

New screening methodologies and new effective therapies are leading to an increasing number of multiplex pilot studies for LSD-NBS, but results are difficult to compare and is difficult to draw definitive conclusions (only Pompe (2015), MPS I (2016) and MPS II (2022) included in RUSP).

# Why NBS for Fabry Disease?

Pan-ethnic, multisystemic, X-linked genetic disease, that affects both male and female, and can lead to severe clinical symptoms

Available therapy, more effective if started at the time of appearance of the first clinical signs

First clinical symptoms are frequently heterogeneous and non-specific, originating diagnostic delays (specially significant for female patients)

Available technology:

Fluorometry (not multiplexable), immune quantification (no commercially available specific antibodies), DMF, ms/ms and LC-ms/ms (multiplexable, commercially available substrates and internal labeled standards)

# NBS for Fabry Disease

## 1st pilot study (2003-2005) - Piedmont region (Italy)

High FD prevalence (1: 3100 male patients)

High frequency of FD late-onset variant

## Taiwan pilot study (2006-2008)

High FD prevalence (1: 1250 male patients)

High frequency of FD late-onset variant, associated with a frequent mutation: IVS4 + 919G>A

## In 2008, FD was nominated for RUSP advisory (US), but not recommended:

- Low test sensitivity for female heterozygotes
- Identification of high prevalence of late-onset variants
- Cost / effectiveness / invasiveness / immunologic response to treatment
- Few prospective FD-NBS and treatment studies

Vincenza Gragnaniello et al., IJNS, 2023

# Results from pilot studies for FD-NBS

Until 2023, more than 20 pilot studies

Estimated prevalences: > 1:2,000 to < 1:15,000

Vincenza Gragnaniello et al., IJNS, 2023

**Europe: few studies, the largest in Northeastern Italy (2015-2021):**

173,342 NB (ms/ms, enzymatic assay)

Vincenza Gragnaniello et al., Biomolecules, 2021

No female patients detected

22 male patients (1:7,879 NB; 1:4,068 males)

13 with pathogenic late-onset mutations (1: 6,883 males)

9 with VUS or benign variants

**US: several studies (7 different states, different approaches):**

Sarah Viall, Hum Mol Metab Rep, 2025

**Ex. Oregon (2018-2023)**

202,729 NB (DMF and ms/ms)

766 positive cases for genetic analysis (2TT - GLA sequencing)

66M + 13F with GLA variants referred for clinical evaluation and confirmation tests

8 male and 4 female confirmed positive (pathogenic or likely pathogenic variants)

No patient started therapy / 50 NB have inconclusive results (possible late onset)

# Results from pilot studies for FD-NBS

**Asia: Taiwan (4), Japan (3), China (1)**

**Ex. Taiwan (from 2006, fluorometry, ms/ms, genetic analysis for 21 selected variants )**

Very high frequency of later-onset IVS4+919G>A (1: 1,600 males)

DNA-based NBS implemented to overcome the high false negative rate in females (IVS4+919G>A)

Vincenza Gragnaniello et al., IJNS, 2023

**South America: Brazil (3), Mexico**

**Ex. Brazil**

20,066 NB (Bahia)

Three-tier approach with integrated analysis: ms/ms-enzyme analysis, LC-ms/ms Lyso-Gb3, NGS)

3 positive cases for low enzyme activity: 1 with high Lyso-Gb3 and a late-onset *GLA* variant, 1 with normal Lyso-Gb3 and one inconclusive variant, 1 with high Lyso-Gb3 and no *GLA* variants identified.

Authors conclude a good performance of this 3 tier strategy, but this is a recent study, including a low number of NB.

Francyne Kubasky et al., Mol Genet Metab, 2023

# Conclusions from FD-NBS pilot studies

-Several studies were performed but it is difficult to compare the results:

- ✓ Different screening techniques and approaches
  - DMF and ms/ms multiplex LSD-enzyme activity assays are currently the preferred 1<sup>st</sup> tier methods, but cut-off definition is critical (low rate of female patients identification Vs. high false positive rate)
  - Different cut-offs (fixed or variant) and frequently changed during the study
  - 2<sup>nd</sup> and 3<sup>rd</sup> tier tests approaches (NGS – may be inconclusive due to VUS; Lyso-Gb3 – late onset and female patients may be missed)
- ✓ Different criteria to define positive cases, leading to variant prevalence estimates (resulting from the identification and different interpretation of VUS and very rare novel private mutations)
- ✓ Geographical and ethnical variations (different genetic backgrounds lead to different results)
- ✓ Short time to evaluate clinical outcomes (late-onset forms)

# marks for FD-NBS

**agnosis and treatment** (frequent diagnosis delay, specially in woman)

**ds on DBS, but**

y (high false positive rate, high recall rate, high number of anxious families, high

)

y for female patients (may lead to even higher diagnostic delay if NBS is negative)

suitable for 2<sup>nd</sup> TT : **enzyme ratios** (need more data), **molecular analysis**

, difficult to evaluate due to frequent detection of VUS, unclassified and benign

**3** (may not be reliable in neonatal period, female and late-onset patients, more

t-analytical tools (more data and collaborations needed)

**ents, but**

cost, effectiveness, immunologic responses and invasiveness of therapeutic

# marks for FD-NBS

**ation** is puzzling due to challenging clinical prognosis and possible missed female

ines for positive cases definition (diagnostic confirmation). Inconclusive cases  
burden for healthcare systems and families.

prevalence estimations are probably too low

et FD may be an important unrecognized genetic disease

**n high number of late-onset patients** raises ethical concerns (represents a  
len to the families)

of **FD natural history** is necessary (poorly understood for late-onset forms)

udies are needed to evaluate clinical outcomes of positive screening cases

can identify new undiagnosed patients and can help to clarify pathogenicity of novel



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