FirstSteps®
NEWBORN GENOME SCREENING

P. Tsipouras,[1] T. Loupis[2], P. Moulos[3], E. Clemente[4], A. Psarris[5], A. Pampanos[5], A. Malamidou[9] ,C. Karasmani[5], M. Koutsogianni[1], E. Stratiki[5],M. Klimatsoudaa[6], S. Kriara[6], E. Babacheva[7], D. Gialambrinou[7], M. Lithoxopoulou[7], T. Xefteris [9] ,M.Reese[8] ,G. Grimbizis[7], C. Tsakalidis[7], A. Daponte[6], G. Daskalakis[5], D. Thanos[2]
FirstSteps[1], BioMedical Research Foundation, Academy of Athens[2], Greece, BSRC 'Alexander Fleming'[3], Vari, Greece, Illumina Research Laboratory[4], Cambridge UK, Alexandra Hospital- National and Kapodistrian University of Athens[5], Greece, University of Thessaly[6], Larissa Greece, Papageorgiou Hospital-Aristotle University[7], Thessaloniki, Greece, Fabric Genomics, Inc.[8]Oakland, CA, USA, Euro Genetica[9], Thessaloniki, Greece.

Introduction

Newborn genome screening represents a transformative shift in healthcare delivery by enabling the early identification of infants at risk of developing serious genetic diseases that are amenable to timely therapeutic intervention. Several national and international initiatives have been launched to evaluate its feasibility, clinical utility, societal acceptance, and overall benefit to healthcare systems.

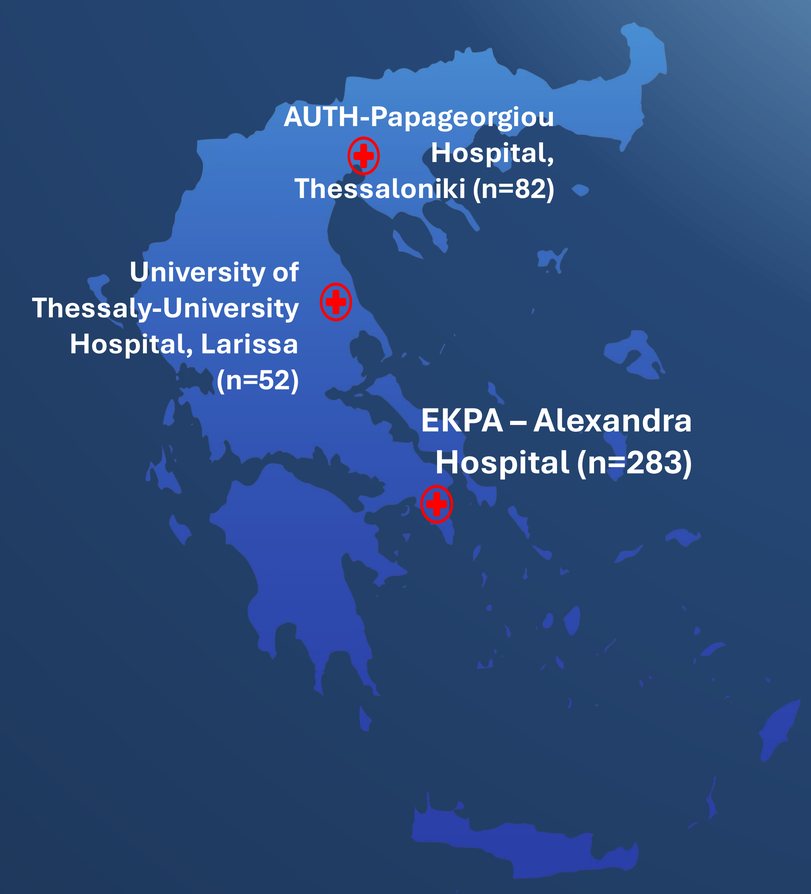
FirstSteps is a multiphase, IRB-approved initiative launched in Greece in mid-2023 to explore these dimensions within the national healthcare context. Phase I involved three University Hospitals and enrolled 417 neonates following informed parental consent. To support informed decision-making, parents received printed educational materials and short explanatory videos, provided either during the third trimester of pregnancy or immediately after birth.

Methods

Prior to hospital discharge, a heel-prick blood sample was collected on filter paper. DNA was extracted from the dried blood spots (DBS) cards and subjected to whole-genome sequencing (WGS) at 30X coverage.

Data analysis targeted a list of 385 genes associated with 504 genetic diseases for which credible therapeutic interventions are available. Only pathogenic (P) and likely pathogenic (LP) variants were considered reportable. All participating families received an individualized test report.

Enrollment Sites (n=417)



Results

385 Genes Tested

417 Babies Enrolled

5.5% screen positive

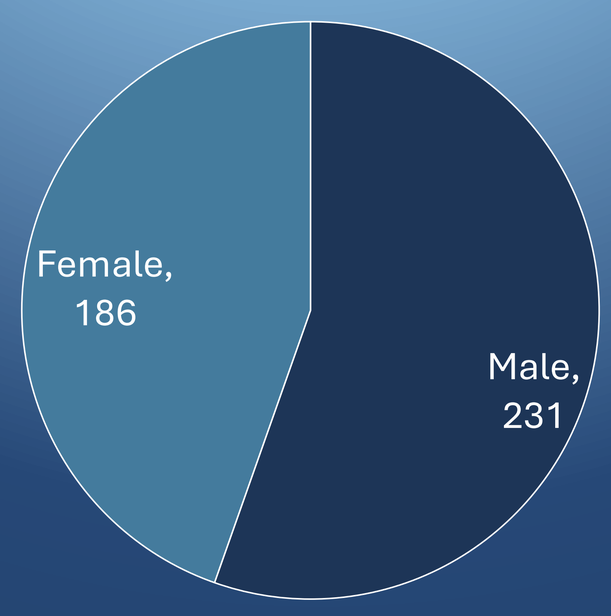
Pathogenic or likely pathogenic (P/LP) variants were identified in the following genes:

F8, G6PD, TCF3, CYP21A1, SCN5A, RYR1, KCNQ1, CFTR, and SCN2A

Orthogonal testing was performed to validate initial findings and included: (i) long-read sequencing (Oxford Nanopore Technologies) to establish allele phase in three instances involving genes linked to autosomal recessive conditions; (ii) Sanger sequencing of family trios to confirm zygosity in 14 cases; (iii) a sweat test in one case; (iv) immunophenotyping in one case; and (v) hematological testing in two cases. Five screen-positive cases remain pending final confirmation.

To date, **eight children have been confirmed as true positive.** Of these, six were diagnosed with **G6PD** deficiency, one with **Hemophilia A** (F8 deficiency), and one homozygous for a **CFTR mutation** (CFSPID). All eight families received genetic counseling and were referred to pediatric specialists.

Total Number Enrolled n=417



Sequencing & Analysis

Sample Preparation and DNA Extraction:
Sample Type: 6 punches from DBS, each 3.2 mm in diameter.
Extraction Kit: Illumina Lysis Reagent Kit (PN 20042221).
Protocol: Manual extraction using optimized conditions.
Quantification: DNA quantified using Quant-iT Broad Range (BR) assay.
DNA Input: Entire extracted DNA used for library preparation.

Library Preparation:
Kit Used: Illumina DNA PCR-Free Library Prep Kit (96 Samples, PN 20041795).
Indexing: Illumina DNA/RNA UD Indexes Set B (PN 20091657).
Automation Platform: Hamilton liquid handler.

Library Quality Control:
qPCR QC: Quantabio sparQ Library Quantification Kit (PN 65210-500).
Concentration QC: Qubit ssDNA Assay Kit (PN Q10212).

Sequencing:
Instrument: NovaSeq X.
Reagents Used:
NovaSeq X Series 10B Reagent Kit (300 Cycle, PN 20085594).
NovaSeq X Series 25B Reagent Kit (300 Cycle, PN 20104706).
Software: Sequencing System Software Suite v1.3.0.
Data Analysis:
Pipeline - cloud-based:
BCL Convert v 2.7.0
DRAGEN Germline v4.3.13

Conclusion

Newborn genome screening has the potential to redefine preventive medicine. The FirstSteps Phase I study was designed to assess feasibility, and our initial findings demonstrate that the number of infants potentially identifiable with a treatable genetic condition is at least an **order of magnitude higher** than those detected through the current national biochemical screening program (33 disorders).

Our results underscore that careful selection of gene–disease pairs, combined with established clinical care pathways, are critical success factors. Ensuring health equity, robust data protection, and capacity-building for healthcare professionals will be essential for sustainable national deployment.

Lead Investigator: P. Tsipouras, MD
Email: petros.tsipouras@beginnings.gr | Website: firststeps-ngs.gr
Presented at ICoNS 2025, London, UK