nature medicine

Article

https://doi.org/10.1038/s41591-024-03465-x

Population-based, first-tier genomic newborn screening in the maternity ward

Received: 22 July 2024

Accepted: 12 December 2024

Published online: 28 January 2025

Check for updates

François Boemer[1,10] ✉, Kristine Hovhannesyan[2,10], Flavia Piazzon[2], Frédéric Minner[2], Myriam Mni[2], Valérie Jacquemin[2], Davood Mashhadizadeh[3], Noor Benmhammed[4], Vincent Bours[5], Adeline Jacquinet[5], Julie Harvengt[5], Saskia Bulk[5], Vinciane Dideberg[5], Laura Helou[6], Leonor Palmeira[6], Tamara Dangouloff[4], BabyDetect Expert Panel* & Laurent Servais[4,7]

The rapid development of therapies for severe and rare genetic conditions underlines the need to incorporate first-tier genetic testing into newborn screening (NBS) programs. A workflow was developed to screen newborns for 165 treatable pediatric disorders by deep sequencing of regions of interest in 405 genes. The prospective observational BabyDetect pilot project was launched in September 2022 in a maternity ward of a public hospital in the Liege area, Belgium. In this ongoing observational study, 4,260 families have been informed of the project, and 3,847 consented to participate. To date, 71 disease cases have been identified, 30 of which were not detected by conventional NBS. Glucose-6-phosphate dehydrogenase deficiency was the most frequent disorder detected, with 44 positive individuals. Of the remaining 27 cases, 17 were recessive disorders. We also identified one false-positive case in a newborn in whom two variants in the *AGXT* gene were identified, which were subsequently shown to be located on the maternal allele. Nine heterozygous variants were identified in genes associated with dominant conditions. Results from the BabyDetect project demonstrate the importance of integrating biochemical and genomic methods in NBS programs. Challenges must be addressed in variant interpretation within a presymptomatic population and in result reporting and diagnostic confirmation.

Every year, thousands of children are born with rare genetic diseases that may lead to death or lifelong disability[1]. Newborn screening (NBS) has been used for decades to identify treatable conditions before the onset of the first symptoms to allow timely interventions that can prevent or minimize long-term health effects. Traditionally, NBS involves collecting a few drops of blood immediately after birth and analyzing this sample by biochemical methods to detect the presence of specific biomarkers. The inclusion of new conditions into an NBS program is driven by criteria formulated by Wilson and Jungner[2] in 1968. The criteria include the existence of an effective treatment and a reliable and cost-effective analytical method.

Recent technological advances have led to the identification of the genetic causes of several diseases, and the rate of introduction of new therapies for rare diseases has remarkably increased in the past decade[3]. Spinal muscular atrophy and severe combined immunodeficiency are examples of diseases for which new treatments are now available. Importantly, these treatments are most effective if initiated before symptoms appear[4–6]. The US Food and Drug Administration estimates that by 2025, there will be 10–20 new cell and gene therapy

A full list of affiliations appears at the end of the paper. ✉e-mail: f.boemer@chuliege.be

Table 1 | Characteristics of the newborn population

Characteristics	*n*	(%)
Sex		
Male	1,957	50.9
Female	1,890	49.1
Birth weight (g)		
<2,500	478	12.4
2,500–4,000	3,155	82.0
>4,000	214	5.6
Gestational age (weeks)		
<37	497	12.9
37–38	964	25.1
39–40	2,092	54.4
>40	294	7.6

approvals per year[7], and it is expected that early or presymptomatic administration of treatments will be correlated with higher life expectancy, avoidance of severe disabilities and fewer complications.

The rarity and lack of medical awareness of rare genetic diseases often lead to a long diagnostic journey, as biomarkers that can be detected by biochemical assays have not been identified for many rare disorders. This has prompted a growing interest in expanding NBS by integrating genomic technologies[8–12]. In September 2022, we launched the BabyDetect project (ClinicalTrials.gov identifier: NCT05687474; www.babydetect.com) to explore the feasibility and acceptability of a population-based, first-tier genomic NBS using targeted next-generation sequencing (tNGS). We report here the results of the first 18 months of this ongoing observational study, which was conducted in a single maternity ward in southern Belgium.

Results

Screened population and samples

From September 2022 to the end of April 2024, the families of 4,260 neonates were informed of the BabyDetect trial. A total of 3,847 neonates were enrolled, corresponding to a 90% acceptance rate. Most (53%) of the parents who opted not to enroll their baby in the study did not disclose a reason for their refusal. Among those who were more forthcoming, the primary rationale was that they deemed the test unnecessary considering that the family and siblings were healthy, the pregnancy had proceeded smoothly or the child appeared to be in good health[13]. The characteristics of the newborns enrolled in the study are presented in Table 1. Of the 3,847 samples analyzed, 84 (2.2%) were retested because of technical issues. The main reasons for testing failures were sample cross-contamination ($n = 16$), sequencing workstation failure ($n = 48$) and poor library quality ($n = 20$).

Variant filtering and review

The list of genes included in BabyDetect target panel is shown in Fig. 1. Zygosity criteria for variant reporting are outlined in Fig. 2. Between 4,000 and 11,000 variants were inferred for each neonate. A dedicated classification tree on the Alissa Interpret platform was used to automatically process variants. The sorting algorithm consisted of a sequence of filters and output bins with optional labels and scores, incorporated into a decision tree topology. The tree allowed us to systematically triage and classify variants. Benign and likely benign variants and variants of unknown significance (VUS) were discarded by the tree, and pathogenic or likely pathogenic genome variants were flagged for manual review before reporting. To comply with the requirement for actionable screening, we report only variants with genotypes known to be associated with a disease. Figure 3 summarizes the applied filtering criteria.

After filtering, flagged samples were manually reviewed to validate variant classification as pathogenic or likely pathogenic and to rule out any potential conflicting interpretations before reporting. This variant review included the American College of Medical Genetics and Genomics interpretation using the Franklin[14,15] and VarSome[16] tools, an extended literature review, and correlation with biochemical results when available. Samples were considered negative if no consensus on the variant was found among the ClinVar, VarSome and Franklin databases.

Positive screening cases

In this ongoing observational study, 3,847 neonates have been tested thus far. After variant filtering, 1% of screened samples required manual review, of which 71 were identified as positive cases for a pathogenic or likely pathogenic variant. Among those neonates, no issues related to discrepancies between phenotypic and genetic sex were observed. Of the positive cases, 44 neonates were identified to have glucose-6-phosphate dehydrogenase (G6PD) deficiency. The positive cases are summarized in Table 2. Nine heterozygous variants were detected in genes associated with conditions that can be inherited in a dominant manner: familial exudative vitreoretinopathy in one neonate, maturity-onset diabetes of the young 13 in one neonate, cardiomyopathy due to a mutation in *MYBPC3* in two neonates and cardiomyopathy due to a mutation in *MYH7* in five neonates. Eighteen neonates were identified to have recessive disorders, including two with glycogen storage disease 1b/c, one with Shwachman–Diamond syndrome, two with hemophilia A, two with hemophilia B, five with cystic fibrosis, one with phenylketonuria, two with partial biotinidase deficiency, one with short-chain acyl-CoA dehydrogenase deficiency, one with carnitine palmitoyltransferase 2 (CPT2) deficiency and one with two class 5 variants in the *AGXT* gene.

We also recorded one false-negative case in a neonate who was referred for cholestasis, jaundice and skin ichthyosis. As part of the diagnostic evaluation, whole-exome sequencing (WES) analysis identified a nonsense c.1030C>T (p.Arg344*) homozygous variant in the *TJP2* gene that was reported as pathogenic given the clinical context. This alteration was detected in the BabyDetect sequencing data, but this variant was not present in our curated variant list and in ClinVar and, to our knowledge, had not been described previously. Consequently, the variant was not flagged for manual review by our sorting tree and was not reported by our workflow. This variant has now been added to our managed variant database.

Seventeen cases were flagged by the filtering tree but were not subsequently reported. These cases included 16 newborns with the benign homozygous Duarte variant c.940A>G (p.Asn314Asp) in the *GALT* gene. All had galactose concentrations within normal limits based on routine NBS, and the BabyDetect results were thus not reported. One neonate was also identified to have the c.1397C>G (p.Ser466*) and c.3209G>A (p.Arg1070Gln) variants in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*). A comprehensive literature review revealed that these variants are frequently reported in a complex *cis*-segregating allele[17,18]. The level of immunoreactive trypsin in this neonate was also far below the cutoff for reporting. Thus, we decided not to report these variants to the attending pediatrician.

Follow-up of positive screening cases

Of the 71 positive cases reported by BabyDetect, 41 cases were identified through conventional NBS (Fig. 4). Among the 30 cases not identified by standard NBS, 10 were G6PD deficiency cases. Measurement of G6PD activity in whole blood confirmed mild deficiencies in all these babies. Patients with G6PD deficiency do not require interventional care unless they experience a hemolytic crisis. However, preventive measures have been taken for the 44 newborns identified to have G6PD deficiency by providing the parents with a list of drugs, chemicals and foods likely to trigger oxidative stress and whose consumption should,

https://doi.org/10.1038/s41591-024-03465-x

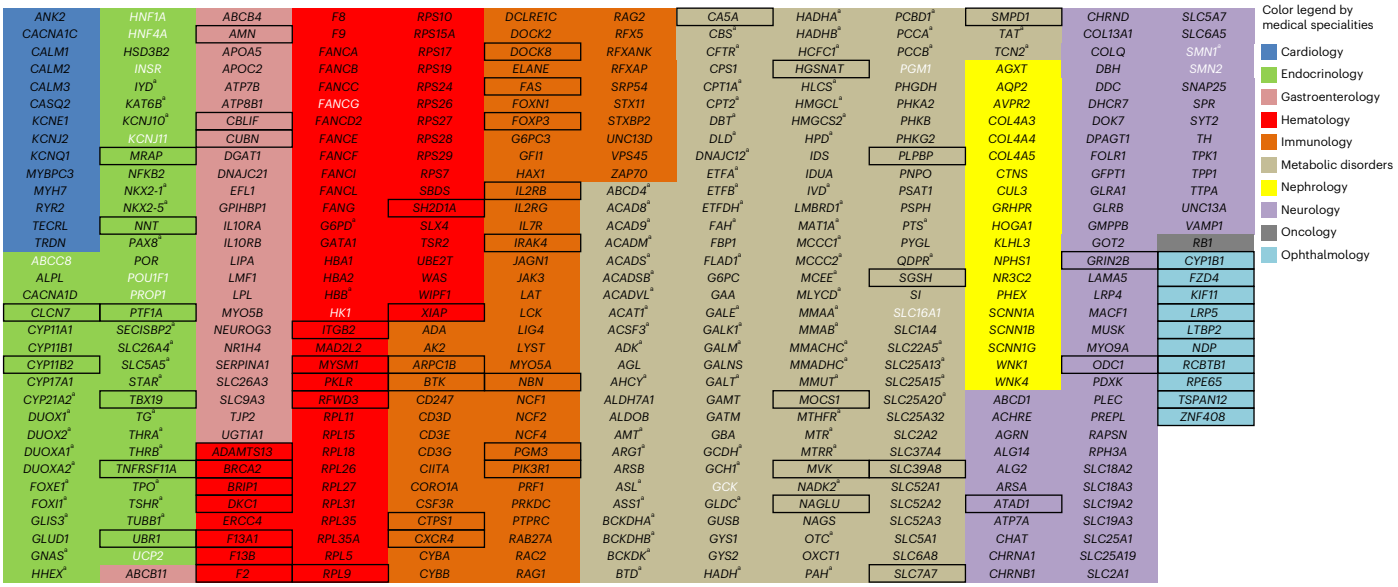

Fig. 1 | List of genes included in the BabyDetect target panel. Boxed genes were added to version 2.0 of the panel, whereas genes in white font were removed from version 1.0. Genes marked with a superscript letter ([a]) are associated with a disorder covered by our conventional NBS.

therefore, be avoided. These babies are followed up by community pediatricians.

In one newborn suspected of having CPT2 deficiency, the condition was also not detected by biochemical NBS. The two variants identified in the *CPT2* gene (c.1339C>T and c.1436A>T) were suggestive of a myopathic form of this deficiency. The result was reported to the pediatrician, and the baby was referred for further metabolic testing. The diagnosis was confirmed by measuring CPT2 activity in cultured cells from the patient. The neonate had a CPT2 activity of 2.6 nmol min^{-1} per mg protein, notably lower than the reference range of 9–22.6 nmol min^{-1} per mg protein. The deficiency was also confirmed by acylcarnitine profiling performed on a plasma specimen, which showed a moderate increase in long-chain acylcarnitines compared to the reference range. Conventional NBS analysis is known to have poor sensitivity for CPT2 screening[19]. This neonate was hospitalized for rhabdomyolysis attacks and myoglobinuria. The availability of BabyDetect results allowed for rapid and appropriate care.

One neonate with a homozygous *CFTR*:c.1865G>A variant, known to be prevalent in African populations and on Reunion Island and associated with a broad spectrum of cystic fibrosis-related phenotypes[20], was also identified. Conventional NBS tests for cystic fibrosis use a two-tier protocol: the first-tier assay measures immunoreactive trypsin, and the second-tier assay involves *CFTR* genotyping. Reporting of the *CFTR* genotyping results is restricted to the 12 most frequent variants found in the Belgian population (Extended Data Table 1). As the *CFTR*:c.1865G>A variant is not one of these variants and because the immunoreactive trypsin level in the patient was far below the positivity threshold, the clinical expert who evaluated the BabyDetect results did not recall the baby for further evaluation.

Two neonates were identified to have hemizygous variants in the *F8* gene. Factor VIII activity measured in fresh plasma samples confirmed mild (activity: 48%) and moderate (activity: 21%) hemophilia A in these neonates. These patients would benefit from the preventive use of desmopressin in preoperative settings to reduce the risk of bleeding complications. The two neonates with glycogen storage disease b/c were twin sisters. After the diagnosis was confirmed, they were immediately placed on a restricted diet. Treatment with empagliflozin was initiated at 8 months of age to prevent neutropenia. One case of *MYH7*-related cardiomyopathy with the heterozygous variant *MYH7*:c.4498C>T was noteworthy. During the confirmatory evaluation, a familial investigation revealed that the father exhibited signs of undiagnosed cardiac hypertrophy. None of these patients were treated with innovative therapies.

The neonate carrying two class 5 variants in the *AGXT* gene (c.33dupC and c.332G>A) was demonstrated to be a false-positive case. Segregation analysis of parental DNA showed that the father carries neither variant and the mother carries both mutations. The mother showed no symptoms of hyperoxaluria.

Turnaround time

The average turnaround time for the BabyDetect screening was calculated as the average of the intervals between the consent date and the variant interpretation date. Goldcards were processed in batches of 96 samples. As around 50 neonates were enrolled per week, analyses were run every 2 weeks. We observed a notable improvement in our average turnaround time over the 18 months of the study to date. The average turnaround time for the first 300 samples analyzed was 64 days (s.d. 33 days), whereas that for the last 300 samples was 51 days (s.d. 10 days). When a conventional NBS comparator was not available, reanalysis took an average of 3 weeks.

Cost

The cost per sample of screening for 165 diseases in the context of this study was 365 euros, which was entirely covered by study funds. This cost does not include material depreciation, overhead, license for secondary analysis (as bioinformatics was conducted on the Humanomics program developed in-house) or follow-up of positive cases.

Discussion

Through the prospective BabyDetect pilot project, we demonstrated the feasibility of a genomic NBS approach at a midscale level. The rapid development of innovative therapies for severe genetic conditions, which cannot be diagnosed by current NBS assays, underlines the need to incorporate genetic testing into NBS. Accordingly, several large prospective studies of genomic NBS have been launched across North America, Europe and Australia to assess the acceptability and feasibility of a first-tier genomic NBS approach[12,21]. To identify affected babies, these pilot trials have implemented tNGS, WES or whole-genome sequencing (WGS) approaches, and the genes queried vary widely between programs[21,22].

https://doi.org/10.1038/s41591-024-03465-x

Table 2 | Positive cases of diseases detected in the BabyDetect study

Case no.	Disorder	Sex	Genotype	Conventional NBS	Conventional NBS result (if available)	Confirmatory result, follow-up	Treatment
1	G6PD deficiency	Female	*G6PD*:c.[292G>A; 466A>G]–c.[292G>A; 466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
2	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
3	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
4	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
5	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
6	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
7	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
8	G6PD deficiency	Male	*G6PD*:c.1450C>T	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
9	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
10	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
11	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
12	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
13	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
14	G6PD deficiency	Male	*G6PD*:c.494A>C	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures

https://doi.org/10.1038/s41591-024-03465-x

Table 2 (continued) | Positive cases of diseases detected in the BabyDetect study

Case no.	Disorder	Sex	Genotype	Conventional NBS	Conventional NBS result (if available)	Confirmatory result, follow-up	Treatment
15	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
16	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
17	G6PD deficiency	Female	*G6PD*:c.[292G>A;466A>G]–c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
18	G6PD deficiency	Female	*G6PD*:c.[292G>A;466A>G]–c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
19	G6PD deficiency	Male	*G6PD*:c.653C>T	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
20	G6PD deficiency	Male	*G6PD*:c.653C>T	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
21	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
22	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
23	G6PD deficiency	Female	*G6PD*:c.653C>T–c.1093G>A	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
24	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
25	G6PD deficiency	Male	*G6PD*:c.494A>C	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
26	G6PD deficiency	Male	*G6PD*:c.653C>T	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
27	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
28	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures

https://doi.org/10.1038/s41591-024-03465-x

Table 2 (continued) | Positive cases of diseases detected in the BabyDetect study

Case no.	Disorder	Sex	Genotype	Conventional NBS	Conventional NBS result (if available)	Confirmatory result, follow-up	Treatment
29	G6PD deficiency	Male	*G6PD*:c.1058T>C–c.466A>G	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
30	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
31	G6PD deficiency	Female	*G6PD*:c.[292G>A;466A>G]–c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
32	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
33	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
34	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Positive	G6PD activity < 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
35	Biotinidase deficiency	Female	*BTD*:c.1270G>C–c.1308A>C	Positive	Biotinidase activity = 31.5% (RR > 47%)	Confirmed by NGS and decreased biotinidase activity in serum, under conventional NBS follow-up	Standard of care: biotin administration
36	Biotinidase deficiency	Male	*BTD*:c.535G>A–c.1270G>C	Positive	Biotinidase activity = 30.5% (RR > 47%)	Confirmed by NGS and decreased biotinidase activity in serum, under conventional NBS follow-up	Standard of care: biotin administration
37	Cystic fibrosis	Male	*CFTR*:c.1521_1523delCTT–c.1521_1523delCTT	Positive	Positive IRT + CFTR analysis	Under conventional NBS follow-up	Standard of care[a]
38	Cystic fibrosis	Male	*CFTR*:c.1521_1523delCTT–c.1521_1523delCTT	Positive	Positive CFTR analysis	Under conventional NBS follow-up	Standard of care[a]
39	Cystic fibrosis	Female	*CFTR*:c.1521_1523delCTT–c.1521_1523delCTT	Positive	Positive IRT + CFTR analysis	Under conventional NBS follow-up	Standard of care[a]
40	Cystic fibrosis	Female	*CFTR*:c.1521_1523delCTT–c.2657+5G>A	Positive	Positive IRT + CFTR analysis	Under conventional NBS follow-up	Standard of care[a]
41	Mild phenylketonuria	Male	*PAH*:c.1222C>T–c.688G>A	Positive	Phenylalanine = 164 $\mu mol\,l^{-1}$ (RR < 120 $\mu mol\,l^{-1}$)	Confirmed by NGS, under conventional NBS follow-up	Standard of care: restrictive diet
42	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
43	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
44	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures

https://doi.org/10.1038/s41591-024-03465-x

Table 2 (continued) | Positive cases of diseases detected in the BabyDetect study

Case no.	Disorder	Sex	Genotype	Conventional NBS	Conventional NBS result (if available)	Confirmatory result, follow-up	Treatment
45	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
46	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
47	G6PD deficiency	Female	*G6PD*:c.[292G>A; 466A>G]–c.[292G>A; 466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
48	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
49	G6PD deficiency	Male	*G6PD*:c.934G>C	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
50	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
51	G6PD deficiency	Male	*G6PD*:c.[292G>A;466A>G]	Negative	G6PD activity > 30% (RR ≥ 30%)	Reduced G6PD activity measured in red blood cells, under conventional NBS follow-up	Preventive measures
52	Short-chain acyl-CoA dehydrogenase deficiency	Female	*ACADS*:c.596C>T–c.1147C>T	Negative	C4-carnitine = 0.06 μmol l^{-1} (RR < 0.08 μmol l^{-1})	Pending	
53	Cystic fibrosis	Male	*CFTR*:c.1865G>A–c.1865G>A	Negative	IRT = 31 μg l^{-1} (RR < 59.8 μg l^{-1})	Neonate not referred, see the text	/
54	CPT2 deficiency	Male	*CPT2*:c.1339C>T–c.1436A>T	Negative	Long-chain acylcarnitines within normal values	CPT2 activity: 2.6 nmol min^{-1} per mg protein (RR = 9–23 nmol min^{-1} per mg protein)	Standard of care initiated at 5 months of age[b]
55	Hyperoxaluria 1	Female	*AGXT*:c.33dupC–c.332G>A	/	/	Not confirmed—false-positive BabyDetect result	Not applicable
56	Hemophilia A	Male	*F8*:c.396A>C	/	/	Factor VIII: 48% (RR > 50%)	Preventive care in preoperative settings
57	Hemophilia A	Male	*F8*:c.6089G>A	/	/	Factor VIII: 21% (RR > 50%)	Preventive care in preoperative settings
58	Hemophilia B	Male	*F9*:c.1345C>T	/	/	Lost to follow-up	/
59	Hemophilia B	Male	*F9*:c.1024A>G	/	/	Pending	
60	Familial exudative vitreoretinopathy	Female	*FZD4*:c.313A>G	/	/	Fundus of the eye examination planned at 9 months of age	Surveillance
61	Maturity-onset diabetes of the young 13	Male	*KCNJ11*:c.902G>A	/	/	Confirmed by Sanger sequencing, positive familial history	Surveillance
62	Cardiomyopathy, hypertrophic, 4	Male	*MYBPC3*:c.3407_3409delACT	/	/	Pending	

https://doi.org/10.1038/s41591-024-03465-x

Table 2 (continued) | Positive cases of diseases detected in the BabyDetect study

Case no.	Disorder	Sex	Genotype	Conventional NBS	Conventional NBS result (if available)	Confirmatory result, follow-up	Treatment
63	Cardiomyopathy, hypertrophic, 4	Female	*MYBPC3*:c.2618C>T	/	/	Pending	
64	Cardiomyopathy, dilated 1S	Female	*MYH7*:c.4498C>T	/	/	Confirmed by Sanger sequencing, positive familial history	Surveillance
65	Cardiomyopathy, dilated 1S	Male	*MYH7*:c.1750G>A	/	/	Confirmed by Sanger sequencing	Surveillance
66	Cardiomyopathy, dilated 1S	Male	*MYH7*:c.4498C>T	/	/	Pending	
67	Cardiomyopathy, dilated 1S	Male	*MYH7*:c.2572C>T	/	/	Pending	
68	Cardiomyopathy, dilated 1S	Male	*MYH7*:c.1370T>C	/	/	Pending	
69	Shwachman–Diamond syndrome	Male	*SBDS*:c.258+2T>C–c.258+2T>C	/	/	Pending	
70	Glycogen storage disease 1b/c	Female	*SLC37A4*:c.1015G>T–c.1015G>T	/	/	Increased 1,5-anhydroglucitol	Standard of care: restrictive diet; empagliflozin initiated at 8 months of age
71	Glycogen storage disease 1b/c	Female	*SLC37A4*:c.1015G>T–c.1015G>T	/	/	Increased 1,5-anhydroglucitol	Standard of care: restrictive diet; empagliflozin initiated at 8 months of age

In the fifth and sixth columns, a slash indicates that the disease is not included in conventional NBS. RR, reference range; IRT, immunoreactive trypsin. [a]Novel therapies for cystic fibrosis are available in Belgium only to patients aged 2 years and older. [b]Initiation of care was subject to the availability of biochemical results, which were obtained after several months. The baby received standard of care at 5 months of age.

At 18 months after the BabyDetect project was launched in one maternity ward, the acceptance rate of the test (>90%) by families attests to the strong buy-in to genomic NBS by the southern Belgian population. The proportion of positive cases identified (1.8%; 0.8% not identified by conventional NBS) must be understood in the context of the broad gene list covered by the panel and by the inclusion of *G6PD*. G6PD deficiency was by far the most frequent disorder detected, with 44 positive individuals (38 male and 6 female neonates). Thirty-five were diagnosed with moderate deficiencies associated with the common A haplotype (*G6PD*:c.[292G>A;466A>G]); the residual G6PD activity in the identified neonates was between 10% and 60% of the reference levels. Conventional NBS programs usually do not screen for G6PD deficiency, but the *G6PD* gene has been included in most genomic NBS trials[21]. Genomic NBS has the potential to diagnose cases not identified by conventional screening, such as the myopathic form of CPT2 deficiency as illustrated in the identification of an infant during our pilot.

The BabyDetect trial is an ongoing prospective observational study. As part of the diagnosis of positive cases, DNA extracted from an independently collected, fresh sample is analyzed by an external laboratory. For certain cases, confirmatory analysis results are still pending, with some delayed by several months. This accounts for the absence of confirmatory and follow-up information for some newborns. We acknowledge this missing information as a limitation of this ongoing study and note that this highlights the challenges inherent in genomic NBS programs.

For the BabyDetect study, a relatively conservative approach in variant reporting is used. Our gene panel is designed to capture only exons and intron–exon boundaries. Consequently, pathogenic variants located within introns, promoters or untranslated regions are not detected by our approach. Additionally, the methodology is not designed to identify certain genetic alterations, such as copy number variations, large deletions, mosaicism or other complex structural abnormalities (for example, translocations, inversions or intricate genomic rearrangements), which further limits its diagnostic accuracy. We also report only pathogenic and likely pathogenic variants that have a consensual curation in several databases, disregarding VUS. Following variant filtering, 1% of screened samples required manual review. The workload generated by this manual review stems from the limitations of our variant filtering tool in assigning X-linked disorders, as well as the conflicting interpretations of certain variants found in the ClinVar database. Whether or not to report VUS is a subject of debate. In the presymptomatic context of population-based NBS, reporting VUS could overload the variant review process, result in an unmanageable recall rate, increase anxiety and mistrust among the screened populations, and require the allocation of substantial resources for confirmatory testing. The risk of false-negative results is illustrated by a patient with a nonsense homozygous variant in the *TJP2* gene, not reported by BabyDetect, in whom the disease was subsequently diagnosed following symptom onset. As genomic NBS becomes more widely used, new pathogenic variants will be detected, particularly in populations traditionally underrepresented in genetic databases. Continuously populating our curated variant list with newly validated disease-causing variants will improve sensitivity and negative predictive value, enhancing the identification of disorders not covered by conventional NBS. Data sharing between genomic NBS programs and careful documentation of false-negative cases are crucial for this process.

False-positive screening results occurred due to *cis*-located double-heterozygous mutations. Variants with a *cis* configuration are not uncommon in the general population[18,23]. Unless parental blood is collected simultaneously with the collection of the baby's blood, biallelic localization of variants cannot be confirmed without contacting

https://doi.org/10.1038/s41591-024-03465-x

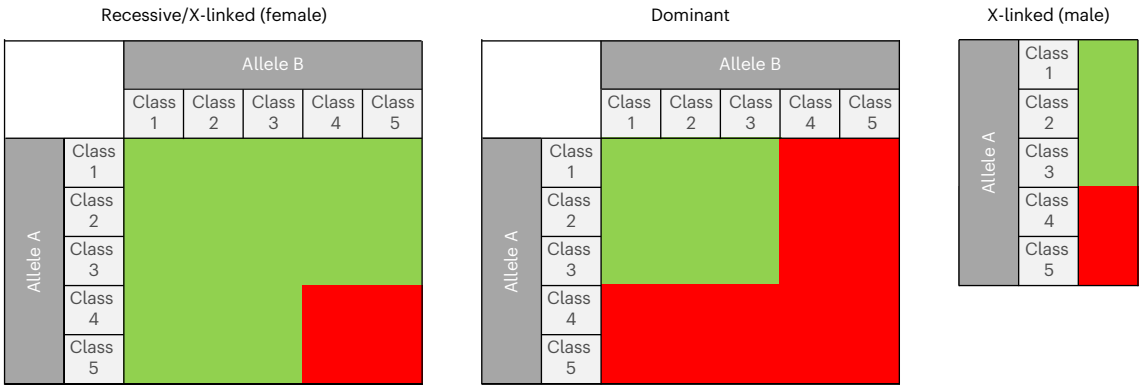

Fig. 2 | Zygosity criteria, based on Mendelian inheritance, for pathogenic (class 5) or likely pathogenic (class 4) variant reporting. Classes 1, 2 and 3 are benign, likely benign and VUS, respectively. Green indicates negative results. Red indicates samples reviewed manually for the accuracy of variant annotations.

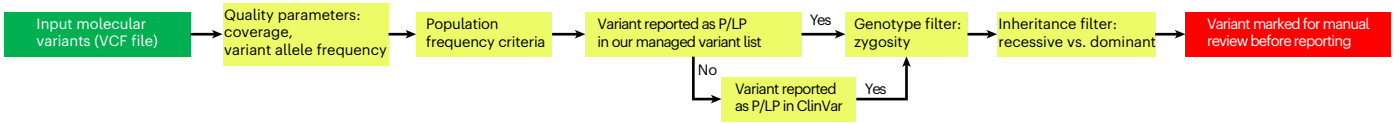

Fig. 3 | Variant filtering criteria. P, pathogenic; LP, likely pathogenic.

the parents. We do not collect blood samples from parents at the time of blood sample collection in neonates owing to logistical hurdles (for example, the need for informed consent and sample storage and tracking), because of the potential of this requirement to decrease the consent rate and because samples are useful only if blood can be collected from both parents. Addressing this limitation will require future genomic NBS programs to incorporate second-tier tests to ascertain the allelic status of variants (for example, using long-read sequencing technologies).

The biochemical data available through coordination with the conventional NBS program also notably assisted in variant assessment. For example, the correlation of BabyDetect results with biomarker concentrations, such as galactose for the homozygous Duarte variant and immunoreactive trypsin for complex *CFTR* alleles, prevented 17 cases from being reported unnecessarily.

The recall rate of 1 in 54 newborns reported here is challenging to manage in a population-based NBS context. The identification of 33 cases of mild G6PD deficiency raises the question of whether reporting such genotypes is warranted, as 10 of these 33 cases were not identified through conventional screening. Within the BabyDetect framework, members of the expert panel and clinical geneticists convene biannually to review and refine the gene panel. This process involves assessing the inclusion of genes linked to newly approved treatments and addressing the challenges of reporting variants with poorly defined penetrance. As an example, the panel chose to revise the reporting of variants in *MYH7*, *MYBPC3* and *KCNJ11* after the identification of five, two and one babies, respectively. The natural history of *MYH7*- and *MYBPC3*-related cardiomyopathies, which have variable ages of onset, and the phenotypic heterogeneity among members of the same family[24,25] are inconsistent with our disease selection criteria. Consequently, the reporting criteria for *MYH7* and *MYBPC3* variants were revised to restrict reporting to instances in which two variants are identified (either homozygous or possibly compound heterozygous). We removed the *KCNJ11* gene from our panel. These examples highlight that the current variant reporting process, which primarily relies on the mode of inheritance, does not take into account crucial information regarding the penetrance of each variant and the age of symptom onset of each disease within populations. Enhancing the characterization of variants will require support from large-scale, long-term, multigenerational studies.

The identification of disorders with a dominant mode of inheritance raises additional questions. For instance, the detection of a heterozygous *MYH7* variant in a neonate subsequently resulted in the diagnosis of cardiomyopathy in the father. Except in cases of de novo mutations, dominant phenotypes are generally expected to be known within affected families. Moreover, as a public health effort, NBS should not aim to identify mild phenotypes.

The sensitivity and positive predictive value of genomic NBS are challenging to calculate reliably. Although sequencing results are generally accurate, the age of symptom onset and the penetrance of many variants are not well defined, making it difficult to determine whether or not to report positive screening results and making it almost impossible to identify false-negative cases. To partially address this limitation, we have amended our consent form to ask parents to agree to be recontacted when their child reaches 1 year of age; this will allow us to collect information on the child's development. Additionally, we will collect information on the treatment and follow-up of positive cases from the physicians.

Despite promising results, tNGS and WES have several shortcomings, including poor coverage, diversity of captured regions, challenges in variant calling and filtering, lack of consensus on the interpretation of many variants, and the absence of information on whether variants are located *cis* or *trans*. Although WES allows a greater number of diseases to be screened than does tNGS, WES has lower sensitivity and specificity than conventional NBS as a primary screening method for inborn errors of metabolism[8,26]. Implementation of large-scale pilot programs and intergenerational population follow-up are necessary to enhance the accuracy of genomic NBS. The development of guidelines for clinical practice will depend on furthering our understanding of how genomic sequences correlate with pathology. Dominant, epistatic, epigenetic and oligogenic mechanisms or other processes that remain unexplained may cause false-negative results. The parents and medical teams need to be aware of this limitation.

BabyDetect sample preparation is currently performed manually. Managing around 2,000 samples a year requires highly skilled staff to

https://doi.org/10.1038/s41591-024-03465-x

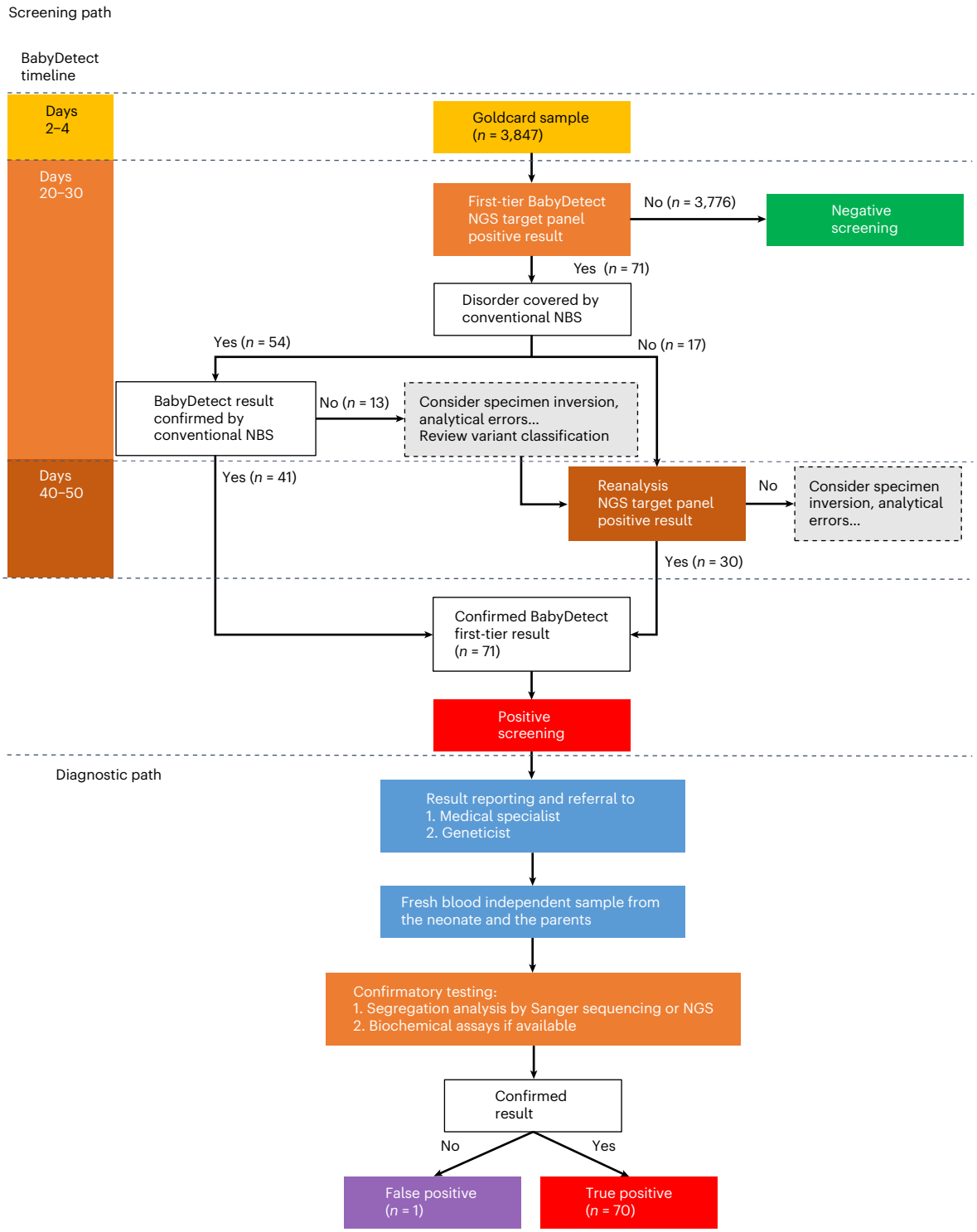

Fig. 4 | BabyDetect screening and diagnostic flowchart. The Goldcard is a dedicated golden filter paper card from LaCAR MDx. The BabyDetect timeline represents the theoretical schedule for BabyDetect result availability.

minimize errors. Extending coverage to screen several thousand babies a year, which would be the case if genomic NBS were extended to all of Belgium, would not be feasible without workflow automation. We made notable strides in reducing our average turnaround time over the 18 months evaluated here, with negative results now available in 51 days, on average, after parental consent is obtained. The improvement in the turnaround time was due to the optimization of the entire process, from double-checking consents before submission to the NBS laboratory to automating DNA extraction with QIAsymphony and accelerating variant inference through our in-house bioinformatics pipeline. Manual assessment of variants is a time-consuming process that adversely affects turnaround time. The number of variants reviewed was limited by precurated lists of pathogenic and likely pathogenic variants, which allowed for higher degrees of automation of the reporting workflow[12]. Finally, with around 50 newborns enrolled each week, analyses were conducted on a biweekly basis. Expanding the project to include a larger population would increase the analysis throughput, thereby notably reducing the turnaround time. Future optimization of methods

and processes could also decrease turnaround time, as demonstrated in NBS for spinal muscular atrophy[5].

Managing the substantial volume of data generated by genomic NBS demands scalable, resilient solutions ensuring data encryption, access control and deidentification to safeguard privacy, adhering to GDPR (General Data Protection Regulation) or HIPAA (Health Insurance Portability and Accountability Act of 1996) regulations. Given that sequencing samples from 3,847 babies by tNGS resulted in 3.5 terabytes of data and raw data must be securely stored for 5 years, expanding the project to hundreds of thousands of babies will inevitably lead to data storage issues.

The cost per sample (365 euros) is notably higher than the 42 euros dedicated by the southern Belgium government to screen for 19 diseases through conventional NBS. However, when considered on a disease basis, the screening of 405 genes is in the same price range as conventional NBS. We expect that technology development and increased volume will considerably decrease these costs over the next several years.

A disadvantage of tNGS is its limited flexibility. For instance, adding 61 new genes to the second version of our panel required a 4-month validation process. To increase the adaptability of the BabyDetect framework, and given that the cost of WES is now comparable to that of tNGS, we plan to transition to WES technology. This shift will eliminate the need for revalidating the entire panel with each new gene inclusion and will streamline the process overall. Looking ahead, we are also considering WGS, as global WGS costs (that is, sequencing, analysis and data storage) are expected to decrease over time. WGS has numerous advantages over WES, including reduced hands-on time for sample preparation and more consistent coverage allowing for easier interpretation of copy number variations or tandem repeat expansions[27].

Although genomic NBS has considerable potential, its practical implementation is undeniably complex. Biochemical and genomic strategies are expected to complement each other in future NBS programs. However, healthcare systems must prepare to handle the increased demand for genetic counseling and follow-up care that will result from the implementation of genomic NBS. Pilot programs such as BabyDetect will help identify and solve clinical, economic, societal, legal and ethical issues that must be addressed before the broad implementation of genomic NBS.

Online content

Any methods, additional references, Nature Portfolio reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41591-024-03465-x.

References

1. Gonzaludo, N., Belmont, J. W., Gainullin, V. G. & Taft, R. J. Estimating the burden and economic impact of pediatric genetic disease. *Genet. Med.* **21**, 1781–1789 (2019).
2. Wilson, J. M. G. & Jungner, G. *Principles and Practice of Screening for Disease* (World Health Organization, 1968).
3. Subbiah, V. The next generation of evidence-based medicine. *Nat. Med.* **29**, 49–58 (2023).
4. Brown, L. et al. Neonatal diagnosis of severe combined immunodeficiency leads to significantly improved survival outcome: the case for newborn screening. *Blood* **117**, 3243–3246 (2011).
5. Boemer, F. et al. Three years pilot of spinal muscular atrophy newborn screening turned into official program in Southern Belgium. *Sci. Rep.* **11**, 19922 (2021).
6. Dangouloff, T. et al. Financial cost and quality of life of patients with spinal muscular atrophy identified by symptoms or newborn screening. *Dev. Med. Child Neurol.* **65**, 67–77 (2023).
7. Gottlieb, S. Statement from FDA Commissioner Scott Gottlieb, M.D. and Peter Marks, M.D., Ph.D., Director of the Center for Biologics Evaluation and Research on new policies to advance development of safe and effective cell and gene therapies. *FDA* www.fda.gov/news-events/press-announcements/statement-fda-commissioner-scott-gottlieb-md-and-peter-marks-md-phd-director-center-biologics (2020).
8. Boemer, F. et al. A next-generation newborn screening pilot study: NGS on dried blood spots detects causal mutations in patients with inherited metabolic diseases. *Sci. Rep.* **7**, 17641 (2017).
9. Bailey, D. B. Jr et al. Early Check: translational science at the intersection of public health and newborn screening. *BMC Pediatr.* **19**, 238 (2019).
10. Kingsmore, S. F. et al. A genome sequencing system for universal newborn screening, diagnosis, and precision medicine for severe genetic diseases. *Am. J. Hum. Genet.* **109**, 1605–1619 (2022).
11. Gold, N. B. et al. Perspectives of rare disease experts on newborn genome sequencing. *JAMA Netw. Open* **6**, e2312231 (2023).
12. Stark, Z. & Scott, R. H. Genomic newborn screening for rare diseases. *Nat. Rev. Genet.* **24**, 755–766 (2023).
13. Dangouloff, T. et al. Feasibility and acceptability of a newborn screening program using targeted next-generation sequencing in one maternity hospital in southern Belgium. *Children* **11**, 926 (2024).
14. da S Rodrigues, E. et al. Variant-level matching for diagnosis and discovery: challenges and opportunities. *Hum. Mutat.* **43**, 782–790 (2022).
15. *Franklin by Genoox* (Genoox, accessed 21 July 2024); franklin.genoox.com/
16. Kopanos, C. et al. VarSome: the human genomic variant search engine. *Bioinformatics* **35**, 1978–1980 (2019).
17. Krasnov, K. V. et al. Localization studies of rare missense mutations in cystic fibrosis transmembrane conductance regulator (CFTR) facilitate interpretation of genotype–phenotype relationships. *Hum. Mutat.* **29**, 1364–1372 (2008).
18. Ivanov, M. et al. Targeted sequencing reveals complex, phenotype-correlated genotypes in cystic fibrosis. *BMC Med. Genomics* **11**, 13 (2018).
19. Yazıcı, H. et al. Experience with carnitine palmitoyltransferase II deficiency: diagnostic challenges in the myopathic form. *J. Pediatr. Endocrinol. Metab.* **37**, 33–41 (2023).
20. Marion, H. et al. The p.Gly622Asp (G622D) mutation, frequently found in Reunion Island and in black populations, is associated with a wide spectrum of CF and CFTR-RD phenotypes. *J. Cyst. Fibros.* **14**, 305–309 (2015).
21. Minten, T. et al. Data-driven prioritization of genetic disorders for global genomic newborn screening programs. Preprint at *medRxiv* https://doi.org/10.1101/2024.03.24.24304797 (2024).
22. Downie, L. et al. Gene selection for genomic newborn screening: moving toward consensus? *Genet. Med.* **26**, 101077 (2024).
23. Chen, T. et al. Genomic sequencing as a first-tier screening test and outcomes of newborn screening. *JAMA Netw. Open* **6**, e2331162 (2023).
24. de Frutos, F. et al. Natural history of *MYH7*-related dilated cardiomyopathy. *J. Am. Coll. Cardiol.* **80**, 1447–1461 (2022).
25. Field, E. et al. Cardiac myosin binding protein-C variants in paediatric-onset hypertrophic cardiomyopathy: natural history and clinical outcomes. *J. Med. Genet.* **59**, 768–775 (2022).
26. Adhikari, A. N. et al. The role of exome sequencing in newborn screening for inborn errors of metabolism. *Nat. Med.* **26**, 1392–1397 (2020).
27. Wojcik, M. H. et al. Genome sequencing for diagnosing rare diseases. *N. Engl. J. Med.* **390**, 1985–1997 (2024).

[1]Biochemical Genetics Lab, Department of Human Genetics, CHU Liege, University of Liege, Liege, Belgium. [2]Human Genetics Laboratory, GIGA-R Institute, University of Liege, Liege, Belgium. [3]Independent researcher, Liege, Belgium. [4]Division of Child Neurology, Reference Center for Neuromuscular Diseases, Department of Pediatrics, CHU Liege, University of Liege, Liege, Belgium. [5]Department of Human Genetics, CHU Liege, University of Liege, Liege, Belgium. [6]Bioinformatics Unit, Department of Human Genetics, CHU Liege, University of Liege, Liege, Belgium. [7]MDUK Neuromuscular Center, Department of Pediatrics, University of Oxford, Oxford, UK. [10]These authors contributed equally: François Boemer, Kristine Hovhannesyan. *A list of authors and their affiliations appears at the end of the paper. ✉e-mail: f.boemer@chuliege.be

BabyDetect Expert Panel

Serpil Alkan[8], Christophe Barrea[8], Paulina Bartoszek[9], Emeline Bequet[8], Hedwige Boboli[8], Romain Bruninx[8], Laure Collard[8], Aurore Daron[8], François-Guillaume Debray[5], Marie-Françoise Dresse[8], Jean-Marie Dubru[8], Iulia Ebetiuc[8], Benoit Florkin[8], Caroline Jacquemart[8], Céline Kempeneers[8], Nadège Hennuy[8], Marie-Christine Lebrethon[8], Marie Leonard[8], Patricia Leroy[8], Angélique Lhomme[8], Jacques Lombet[8], Sabine Michotte[8], Ariane Milet[8], Anne-Simone Parent[8], Vincent Rigo[8], Marie-Christine Seghaye[8] & Sandrine Vaessen[8]

[8]CHR Citadelle, Liege, Belgium. [9]Cliniques Universitaires Saint-Luc, Brussels, Belgium.

Methods

Ethics

The project was approved by the CHU Liege ethics committee (no. 2021/239) and was conducted in accordance with the Declaration of Helsinki. Patients or guardians provided voluntary informed consent to participate in the study, free of coercion or coercive circumstances.

Population

Newborn recruitment was carried out over 18 months in one maternity ward at the public hospital of CHR Citadelle in Liege, Belgium, one of the largest in our area, with approximately 2,500 births annually. From September 2022 to the end of April 2024, a total of 4,260 babies were born at CHR Citadelle. Patients of this hospital reflect the general population of the Liege area, which is highly diverse in terms of ethnic origin and socioeconomic status. The proportion of non-Belgian inhabitants in Liege is 20.38% (versus 10.97% in southern Belgium), and the median income per household is 21,589 euros (14.3% below the southern Belgium average; https://walstat.iweps.be). Consanguinity in this population is uncommon but occasionally observed in some ethnic groups. Information on the sex of the neonates (male or female) was collected to support the interpretation of sex-chromosome sequencing data. This phenotypic sex was provided by the referring pediatrician. Information on gender was not relevant as the study population comprised newborns.

We previously reported the overall study setup and consent process[13]. Briefly, all parents are informed about the project before delivery. Flyers, posters and audiovisual content with information and links to the study website are available in the waiting rooms of the maternity ward and those of gynecologists who support the project. After birth and before sample collection, good clinical practice-certified data managers and trained students collect digital informed consent from parents on a dedicated and secured website. This consent confirms that data could be used for further medical consults and research purposes. Enrollment in the trial is free of charge. For consented babies, a few drops of blood are collected on the Goldcard, a dedicated golden filter paper card (LaCAR MDx), on the first days after birth. Then, testing is performed in our region's conventional NBS reference laboratory.

Selection of genes

The general inclusion criteria for genes incorporated into the initial test panel and the version of the panel implemented after 1 year were as follows: notable consequences for life expectancy or severe disability associated with an untreated disease, disease onset before 5 years of age, strong genotype–phenotype correlation, the existence of a disease-modifying treatment accessible to the diagnosed patients and notable benefit of prompt treatment. Genes with mutations that underlie diseases currently screened in our biochemical panel were also included even if they did not match these criteria (for example, G6PD deficiency). Approval by pediatricians who specialize in the treatment of the disease was mandatory. Screening for a given disease would have been discontinued if a disease-modifying treatment became unavailable (for example, withdrawn from the market or no longer reimbursed, which did not occur) or if there were operational issues that precluded accurate testing. Discussions were held periodically with experts to review the list of disorders and genes in the tNGS panel. We first used a panel targeting 359 genes, including 104 genes coding for disorders currently screened by conventional NBS (Extended Data Table 1) and 255 additional genes coding for defects not amenable to biochemical screening. The panel was reviewed after 1 year of testing; 61 genes were added and 15 were removed, leaving a total of 405 genes. These genes are associated with 165 treatable severe pediatric disorders. The full list of genes included is presented in Fig. 1.

Gene panel-based sequencing

DNA was initially extracted manually from three 3.2-mm dried blood spots using the QIAamp DNA Investigator kit (Qiagen). Currently, DNA is extracted using the QIAsymphony instrument (Qiagen). Target enrichment is performed using Twist Bioscience preparation reagents. Captured regions cover only the coding regions and intron–exon boundaries (~50 base pairs from the intronic borders) of selected genes. Deep intronic variants, promoter and untranslated regions, and homopolymeric regions are not sequenced. With target panel version 2.0, approximately 1.5 Mb are sequenced.

Libraries are sequenced on the NovaSeq 6000 or NextSeq 550 platform (Illumina) with an average read depth coverage of 200×. Sequence alignment onto the GRCh37 (hg19) human reference genome, data quality control and variant inference are performed on the Humanomics (12 September 2024) bioinformatics pipeline developed in the Genetics Department of the CHU Liege following the GATK (Genome Analysis Toolkit) best practices pipeline[28,29]. Briefly, all paired-end reads are mapped to the reference genome, and optical and PCR duplicates are removed. Identification of small nucleotide variants, insertions and deletions, and quality control evaluation are performed with HaplotypeCaller. All values of quality control metrics are stored in a local database for traceability. Raw sequencing data and results are stored in a hospital-grade storage facility that follows the standard policies for redundancy, data integrity and availability, and network security. Computation is performed on the hospital-hosted high-performance computing infrastructure. The Humanomics tool allows identifying single-nucleotide polymorphisms and indels located within exons or at the intron–exon boundary (~50 base pairs of flanking regions). The pipeline does not call copy number variations, large deletions, mosaicism or other structural abnormalities (for example, translocations and inversions).

Variant reporting

Variant annotation, prioritization, classification and interpretation are performed using Alissa Interpret v.5.4.2 (Agilent Technologies), which is a secure variant assessment cloud platform also intended for variant storage. Phenotype-driven interpretation of variants using Human Phenotype Ontology codes is not useful for neonates. Therefore, variant annotation is performed using an internally curated list of genomic variations and the ClinVar database[30]. According to the American College of Medical Genetics and Genomics classification of variants[31], we report only class 4 (likely pathogenic) and class 5 (pathogenic) variants. Benign, likely benign and VUS are disregarded. Additionally, variants not documented in ClinVar or our curated list are not reported. Variants reported in ClinVar are subsequently reviewed with particular caution using the Franklin[14,15] and VarSome[16] platforms, which have an advanced artificial intelligence-driven engine designed to prioritize and interpret variant data. In genes associated with autosomal recessive disorders, the identification of two pathogenic or likely pathogenic variants is necessary to report a positive result for the corresponding disease. For autosomal dominant diseases, the identification of one pathogenic or likely pathogenic variant is considered a positive result. For genes located on the X chromosome, hemizygous identification of pathogenic or likely pathogenic variants in male neonates and homozygous or possible compound heterozygous identification of pathogenic or likely pathogenic variants in female neonates are considered positive results (Fig. 2).

Screening process

Goldcard specimens are registered in the laboratory information system used for the conventional NBS program under the same patient entry, enabling genotype–phenotype correlation assessment for genes covered by both the conventional NBS program and BabyDetect. For disorders not covered by conventional NBS, first-tier positive samples are reanalyzed (from DNA extraction to variant interpretation) to rule out errors in specimen assignment to a particular individual. For disorders included in our conventional NBS program, BabyDetect sequencing data are matched to biochemical results to confirm the result.

Parents were informed that, as is the case for conventional NBS in southern Belgium, no negative reports will be issued, and the test should be considered negative in the absence of a report within 3 months. When BabyDetect identified a disease also identified by conventional NBS, no further action was taken through the BabyDetect program as the baby was managed per standard of care. Positive tests not identified by conventional NBS were communicated by the laboratory to the neonate's pediatrician, the referent specialists of the identified disorder and referent geneticists. Parents were contacted to plan a consultation as soon as possible. At this consultation, a fresh blood sample was collected from the neonate, and blood samples were collected from the parents for segregation analysis. Independent confirmatory testing was performed by Sanger sequencing, tNGS or biochemical assays depending on the disorder (Fig. 4). After the confirmation of a positive screening result, appropriate care was initiated, and parents were recommended to seek genetic counseling.

Outcomes

The study outcomes focused on assessing the acceptability and feasibility of genomic NBS within the studied population. The proportion of parents who provided consent for the proposed screening was meticulously recorded in relation to the total number of mothers approached. The clinical performance of the screening process was rigorously evaluated, with particular attention to the rate of positive findings. Estimates of false-positive and false-negative results were derived through close collaboration with physicians managing the associated conditions. Furthermore, the turnaround time of the screening process was carefully monitored to ensure the timely delivery of results.

Reporting

We report the study results following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines[32].

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

In accordance with the informed consent agreements, the raw sequencing data can be stored for each patient for a period of 10 years. Metadata files are retained with no time limit. The raw sequencing data and metadata files generated in the study cannot be made publicly available because of ethical and data protection constraints. Deidentified data that support the results reported in this article will be made available to suitably qualified researchers through any requests that comply with ethical standards to the corresponding author (F.B., f.boemer@chuliege.be). Data must be requested between 1 and 12 months after the paper has been published, and the proposed use of the data must be approved by an independent review committee identified for this purpose by mutual agreement. Requests will be forwarded by the corresponding author to the identified ethics review committee. Upon acceptance by that committee, deidentified data will be provided by the corresponding author to the applicants through a secured web platform within 2 months. The minimum dataset required to run our code and reproduce results is available via Zenodo at https://doi.org/10.5281/zenodo.13935241 (ref. 29).

Code availability

The Humanomics pipeline used in this article is publicly distributed under GNU Affero General Public License version 3 (https://gitlab.uliege.be/bif-chu/humanomics). The version used for analyses described here (12 September 2024) is available as an official release on the GitLab repository. For traceability and reproducibility concerns, a Zenodo record is provided (https://doi.org/10.5281/zenodo.13935241)[29].

References

28. Van der Auwera, G. A. et al. From FastQ data to high confidence variant calls: the Genome Analysis Toolkit best practices pipeline. *Curr. Protoc. Bioinformatics* **43**, 11.10.1–11.10.33 (2013).
29. Charloteaux, B. et al. Humanomics. *Zenodo* https://doi.org/10.5281/zenodo.13935241 (2024).
30. Landrum, M. J. et al. ClinVar: improving access to variant interpretations and supporting evidence. *Nucleic Acids Res.* **46**, D1062–D1067 (2018).
31. Richards, S. et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet. Med.* **17**, 405–424 (2015).
32. von Elm, E. et al. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet* **370**, 1453–1457 (2007).

Acknowledgements

This work was supported by private and public grants (Sanofi (SGZ-2019-12843), Orchard Therapeutics, Takeda, Léon Frédéricq Foundation) and by a donation from LaCAR MDx. V.B. was funded by a Région Wallonne grant (WALGEMED project 1710180). The funders of this study had no role in study design, data collection, data analysis, data interpretation or writing of the report.
We thank J. Wyatt for the language editing.

Author contributions

F.B. contributed to conceptualization, data curation, formal analysis, investigation, methodology, resources, software, supervision, validation, and the writing, review and editing of the paper. K.H. contributed to data curation, formal analysis, investigation, methodology, software, validation, and the writing, review and editing of the paper. F.P. contributed to methodology, validation (gene panel curation), and the writing, review and editing of the paper. F.M. contributed to formal analysis, resources and software. M.M. contributed to formal analysis and resources. V.J. contributed to data curation, formal analysis, investigation, validation, and the writing, review and editing of the paper. D.M. contributed to funding acquisition and project administration. N.B. contributed resources. V.B. contributed resources and to the writing, review and editing of the paper. A.J. contributed to methodology, validation (gene panel curation), and the writing, review and editing of the paper. J.H. contributed to methodology, validation (gene panel curation), and the writing, review and editing of the paper. S.B. contributed to methodology, validation (gene panel curation), and the writing, review and editing of the paper. V.D. contributed to methodology, validation (confirmation of screening results), and the writing, review and editing of the paper. L.H. contributed to software development (consolidated the bioinformatics pipeline and sequencing results analysis) and the writing, review and editing of the paper. L.P. contributed to software development (consolidated the bioinformatics pipeline and sequencing results analysis) and the writing, review and editing of the paper. T.D. contributed to funding acquisition, methodology, project administration, resources, and the writing, review and editing of the paper. L.S. contributed to conceptualization, funding acquisition, investigation, project administration, resources, supervision, and the writing, review and editing of the paper. The BabyDetect Expert Panel consists of a group of medical specialists who contributed to the selection of genes and are in charge of managing the care of confirmed cases. All authors approved

the paper and agreed to be accountable for the accuracy of the reported findings.

Competing interests

F.B. has consulted for LaCAR MDx and has presented lectures for Novartis and Sanofi. T.D. has presented lectures for Biogen, Roche and Novartis. L.S. is a member of scientific advisory boards or has consulted for Biogen, Novartis, Roche, Illumina, Sanofi, Scholar Rock, LaCAR MDx and BioHaven. The other authors declare no competing interests.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-024-03465-x.

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41591-024-03465-x.

Correspondence and requests for materials should be addressed to François Boemer.

Peer review information *Nature Medicine* thanks Roberto Giugliani and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. Primary Handling Editor: Anna Maria Ranzoni, in collaboration with the *Nature Medicine* team.

https://doi.org/10.1038/s41591-024-03465-x

Extended Data Table 1 | Conventional NBS

Conventional NBS assay	Disorder	Gene	Second-Tier test	Turnaround Time	Confirmatory test
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*ABCD4*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Isobutyrylglycinuria	*ACAD8*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	ACAD9 deficiency	*ACAD9*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Medium Chain Acyl-CoA Dehydrogenase Deficiency (MCAD)	*ACADM*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Short-Chain Acyl-CoA Dehydrogenase Deficiency (SCAD)	*ACADS*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	2-Methylbutyrylglycinuria	*ACADSB*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Very Long Chain Acyl-CoA Dehydrogenase Deficiency	*ACADVL*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Beta-Ketothiolase Deficiency	*ACAT1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*ACSF3*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Hypermethioninemia	*ADK*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Hypermethioninemia	*AHCY*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Glycine encephalopathy	*AMT*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Argininemia	*ARG1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Argininosuccinic Aciduria	*ASL*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Citrullinemia Type I	*ASS1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Maple Syryp Urine Disease (MSUD)	*BCKDHA*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Maple Syryp Urine Disease (MSUD)	*BCKDHB*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Branched-Chain Alpha-Ketoacid Dehydrogenase Deficiency	*BCKDK*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Homocystinuria	*CBS*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Carnitine Palmitoyltransferase Type I Deficiency (CPT1)	*CPT1A*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Carnitine Palmitoyltransferase Type II Deficiency (CPT2)	*CPT2*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Maple Syryp Urine Disease (MSUD)	*DBT*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Dihydrolipoamide Dehydrogenase deficiency	*DLD*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Phenylketonuria	*DNAJC12*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)	*ETFA*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)	*ETFB*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)	*ETFDH*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Tyrosinemia Type I	*FAH*	Succinylacetone	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Multiple Acyl-CoA Dehydrogenase Deficiency (MADD)	*FLAD1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Glutaric Aciduria type I	*GCDH*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Phenylketonuria	*GCH1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Glycine encephalopathy	*GLDC*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Medium/Short-Chain L-3-HydroxyacylCoA Dehydrogenase Deficiency	*HADH*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Long-chain L-3 Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)	*HADHA*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Long-chain L-3 Hydroxyacyl-CoA Dehydrogenase Deficiency (LCHAD)	*HADHB*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*HCFC1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Holocarboxylase Synthase Deficiency	*HLCS*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	3-Hydroxy-3-Methyglutaric Aciduria	*HMGCL*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	3-HMG-CoA Synthase-2 Deficiency	*HMGCS2*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Tyrosinemia Type III	*HPD*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Isovaleric Acidemia	*IVD*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*LMBRD1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Hypermethioninemia	*MAT1A*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	3-Methylcrotonyl-CoA Carboxylase Deficiency	*MCCC1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	3-Methylcrotonyl-CoA Carboxylase Deficiency	*MCCC2*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*MCEE*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Malonic Aciduria	*MLYCD*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*MMAA*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*MMAB*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*MMACHC*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*MMADHC*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*MMUT*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Homocystinuria	*MTHFR*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Homocystinuria	*MTR*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Homocystinuria	*MTRR*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	2,4 Dienoyl-CoA Reductase Deficiency	*NADK2*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Ornithine Transcarbamylase Deficiency	*OTC*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Phenylketonuria	*PAH*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Phenylketonuria	*PCBD1*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Propionic Acidemia	*PCCA*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Propionic Acidemia	*PCCB*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Phenylketonuria	*PTS*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Phenylketonuria	*QDPR*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Carnitine Uptake Deficiency	*SLC22A5*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Citrullinemia Type II	*SLC25A13*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	HHH Syndrome	*SLC25A15*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Carnitine Acylcarnitine Translocase Deficiency	*SLC25A20*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Tyrosinemia Type II	*TAT*	/	3 days	Biochemical testing, Gene sequencing
Amino Acids - Acylcarnitines	Methylmalonic Aciduria	*TCN2*	/	3 days	Biochemical testing, Gene sequencing
TSH	Congenital Hypothyroidism	*DUOX1*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*DUOX2*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*DUOXA1*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*DUOXA2*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*FOXE1*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*FOXI1*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*GLIS3*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*GNAS*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*HHEX*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*IYD*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*KAT6B*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*KCNJ10*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*NKX2-1*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*NKX2-5*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*PAX8*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*SECISBP2*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*SLC26A4*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*SLC5A5*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*TG*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*THRA*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*THRB*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*TPO*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*TSHR*	/	3 days	Thyroid biomarkers on serum, scintigraphy
TSH	Congenital Hypothyroidism	*TUBB1*	/	3 days	Thyroid biomarkers on serum, scintigraphy
17-Hydroxyprogesterone	Congenital Adrenal Hyperplasia	*CYP21A2*	/	3 days	17-Hydroyprogsterone and electrolytes on serum, Gene sequencing
17-Hydroxyprogesterone	Congenital Adrenal Hyperplasia	*STAR*	/	3 days	17-Hydroyprogsterone and electrolytes on serum, Gene sequencing
Immunoreactive Trypsin	Cystic Fibrosis	*CFTR*	*CFTR* genotyping*	3 days	Sweat test, Gene sequencing
Galactose	Galactosemia	*GALE*	/	3 days	Galactose-1P in red blood cells, Urinary Galactitol, Gene sequencing
Galactose	Galactosemia	*GALK1*	/	3 days	Galactose-1P in red blood cells, Urinary Galactitol, Gene sequencing
Galactose	Galactosemia	*GALM*	/	3 days	Galactose-1P in red blood cells, Urinary Galactitol, Gene sequencing
Galactose	Galactosemia	*GALT*	GALT activity	3 days	Galactose-1P in red blood cells, Urinary Galactitol, Gene sequencing
Biotinidase activity	Biotinidase Deficiency	*BTD*	/	3 days	Biotinidase activity on serum, Gene sequencing
Hemoglobin variant	Sickle Cell Disease	*HBB*	/	7 days	Hemoglobin electrophoresis
qPCR SMN1 gene	SMA	*SMN1*	/	3 days	MLPA
G6PD activity	G6PD Deficiency	*G6PD*	/	7 days	G6PD activity on red blood cells

* *CFTR* genotyping is perfomed using Elucigene kit, which allows the identification of 50 *CFTR* variants. Only the 12 most frequent variants in the Belgian population are reported (variant legacy names: F508del, G542X, N1303K, 1717-1G>A, 3272-26A>G, S1251N, A455E, 2789+5G>A, R553X, W1282X, 3849+10kbC>T, R1162X)

nature portfolio

Corresponding author(s): François BOEMER

Last updated by author(s): Dec 9, 2024

Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed
☒	☐ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☒	☐ A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐ The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of all covariates tested
☒	☐ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☒	☐ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐ For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐ Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Digital consents were collected via a secure web interface developed for the purposes of the project by Atracore, a commercial company specializing in digital health intelligence (https://atracore.com). The consent collection interface is available at https://babydetect.atramed.solutions.

Data analysis	Libraries were sequenced on Novaseq 6000 or Nextseq 550 platforms (Illumina). Sequence alignment onto the GRCh37 (hg19) human reference genome, data quality control, and variant inference were performed on the Humanomics v2024.09.12 bioinformatics pipeline developed in the Genetics Department of the CHU of Liège following the GATK best practices (see below for software versions and links to the repositories where data and code are deposited). Briefly, all paired-end reads are mapped to the reference genome (bwa, samtools), and optical and PCR duplicates are removed (elprep). Small nucleotide variants, insertions, and deletions are inferred (GATK) and quality control evaluation is performed (PICARD, mosdepth, somalier). All quality control metrics values are stored in a local database for traceability (MultiQC, ChronQC, MariaDB). Raw sequencing data and results are stored in a hospital-grade storage facility that follows the standard policies for redundancy, data integrity and availability, and network security. Computation is performed on the hospital-hosted high-performance computing infrastructure. The Humanomics tool allows identification of single nucleotide polymorphisms (SNPs) and indels located within exons or at the intron-exon boundary (~50 base pairs of flanking regions). The pipeline does not call copy number variations, large deletions, mosaicism, or other structural abnormalities (e.g., translocations). The Humanomics pipeline used in this article is publicly distributed under GNU Affero General Public License version 3 (https://gitlab.uliege.be/bif-chu/humanomics). The version used for analyses described here (2024.09.12) is available as an official release on the GitLab repository. For traceability and reproducibility concerns, a Zenodo record is provided (https://doi.org/10.5281/zenodo.13739359). Variant annotation, prioritization, classification, and interpretation are performed using a dedicated Alissa Interpret v.5.4.2 (Agilent Technologies) platform. This application is available at https://babydetect.alissa.agilent.com/

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

In accordance with the informed consent, the raw sequencing data can be stored for each patient for a period of 10 years. Metadata files are retained with no time limit. The raw sequencing data and metadata files generated in the study cannot be made publicly available because of ethical and data protection constraints. Deidentified data that support the results reported in this article will be made available to suitably qualified researchers through any requests that complies with clinical study ethical to the corresponding author (FB, email: f.boemer@chuliege.be). Data must be requested between 1 and 12 months after the manuscript has been published and the proposed use of the data must be approved by an independent review committee identified for this purpose by mutual agreement. Requests will be forwarded by the corresponding author to the identified ethics review committee. Upon acceptance by that committee, de-identified data will be provided by the corresponding author to the applicants through a secured web-platform within two months. The minimum dataset required to run our code and reproduce results is available at https://doi.org/10.5281/zenodo.13739359.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	The sexes of the neonates (male or female) were collected to support the interpretation of sex-chromosome sequencing data. This phenotypic sex was provided by the referring pediatrician. Information on gender was not relevant, as the study population comprised newborns. Among the 3,847 neonates, 1,957 were males and 1,890 were females.
Population characteristics	The studied population are neonates. Dried blood spots samples were collected during the first days of life.
Recruitment	Newborn recruitment was carried out in one maternity ward at the public hospital of CHR Citadelle in Liège, Belgium, one of the largest in our area, with approximately 2,500 births annually. From September 2022 to the end of April 2024, a total of 4,260 babies were born at CHR Citadelle. We previously reported the overall study setup and consent process. Briefly, all parents were informed about the project prior to delivery. Flyers, posters, and audio-visual content with information and links to the study website were available in the waiting rooms of the maternity ward and from gynecologists who supported the project. After birth and prior to sample collection, GCP-certified data managers and trained students collected digital informed consent from parents on a dedicated and secured website. Guardians were also asked whether the data of their child could be used for further medical consultation and research purposes. Enrollment in the trial was free of charge. For consented babies, a few drops of blood were collected in the first days after birth on a Goldcard, a dedicated golden filter paper card (LaCAR MDx), and testing was performed in our region's conventional NBS reference laboratory.
Ethics oversight	The project was approved by the CHU Liege ethics committee (n° 2021/239) in accordance with the Declaration of Helsinki.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. Newborn recruitment was carried out in one maternity ward at the public hospital of CHR Citadelle in Liège, Belgium. From September 2022 to the end of April 2024, a total of 4,260 babies were born at CHR Citadelle. All families were informed of the BabyDetect trial, and 3,847 neonates were enrolled.
Data exclusions	No data were excluded from the analysis.
Replication	Prior to the launch of the clinical study in September 2022, the accuracy, sensitivity and concordance of our analytical workflow were validated using the reference sample NA24385-HG002 (Coriell Institute). Analytical sensitivity was calculated at 97.2% for single nucleotide polymorphisms (SNPs) and at 81.5 % for indels. Predictive positive value is 93.7% for SNPs and 59% for indels. The concordance in variant identification among replicates exceeds 95%.
Randomization	Samples were not randomized, as this study was designed as a prospective observational investigation. Positive screening results were communicated to pediatricians and parents.
Blinding	Results were not blinded. Positive results were communicated to pediatricians and parents to ensure appropriate care. The investigators were also informed of the results to support the continuous population of the managed variant database.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☒	☐ Animals and other organisms
☐	☒ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	NCT05687474
Study protocol	Every year, thousands of children around the world are born with rare genetic diseases leading to death or lifelong disability. With technological advancements in the field of genetics and medicine, the rate of introduction of treatments for these rare conditions has grown remarkably. However, timing is of great importance for medication administration. The benefit that can be measured in a patient who has already suffered from a long irreversible degenerative disorder is small and, sometimes, it hardly justifies the cost and the burden of the treatment. Early diagnosis is, thus, of primary importance both to obtain the best effect of the innovative medications and to accelerate their development. Our project methodology includes parental consent, blood sampling of newborns on a filter paper card, DNA extraction, gene panel sequencing, variant inference, variant interpretation and communication of results to pediatricians and parents. Confirmation of screening-positive cases, and their follow-up, enables the sensitivity and specificity of this genomic screening to be evaluated.
Data collection	Newborn recruitment was conducted over an 18-month period in a single maternity ward at CHR Citadelle, a public hospital in Liège, Belgium. The recruitment began on September 1, 2022, and concluded on April 30, 2024.
Outcomes	The study outcomes focused on assessing the acceptability and feasibility of genomic newborn screening within the studied population. The proportion of parents who provided consent for the proposed screening was meticulously recorded in relation to the total number of mothers approached. The clinical performance of the screening process was rigorously evaluated, with particular attention to the rate of positive findings. Estimates of false-positive and false-negative results were derived through close collaboration with physicians managing the associated conditions. Furthermore, the turnaround time of the screening process was carefully monitored to ensure the timely delivery of results.