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Genetic Newborn Screening for Retinoblastoma: A Belgian Initiative Baby Detect

Paulina Bartoszek, MD, François Boemer, PhD,† Kristine Hovhannesyan, PhD,‡ Valérie Jacquemin, PhD,‡ Flavia Piazzon, MD, PhD,‡ Davood Mashhadizadeh, MS,§ Vincent Bours, MD, PhD,‖ Laura Helou, PhD,¶ Vincent Rigo, MD, PhD,# Nadège Hennuy, MD,# Tamara Dangouloff, PhD,** and Laurent Servais, MD, PhD**††*

Abstract: Baby Detect Project, started in September 2022, aimed to create a newborn screening test using targeted next-generation sequencing for all early-onset, treatable, and serious conditions. The elaborated gene panel covers 405 genes, associated with 165 genetic conditions, and includes *RB1*, linked to retinoblastoma, the only oncological disease tested for. Germline *RB1* mutations concern around 50% of all retinoblastoma cases and 100% of the most severe, bilateral cases. Ninety percent of them occur de novo, which delays the diagnosis by about a year with subsequent loss of vision and sometimes the eye itself. Detecting children with germline *RB1* mutation at birth would greatly improve functional and anatomic outcomes, limiting invasive treatments and general anesthesias through early childhood. We discuss herein the novel approach of population screening, the rationale for newborn testing for *RB1* mutations, the incidence of expected cases, the reliability of the test and its costs. The next step is to move to a nation-scale population; this initiative marks a landmark in retinoblastoma patients' care.

Key Words: genomic newborn screening, ; Retinoblastoma, precision medicine, population health

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From the *Department of Ophthalmology, University Hospitals Saint-Luc, Institut Roi Albert II, UCLouvain, Brussels; †Biochemical Genetics Lab, Department of Human Genetics, CHU Liege, University of Liege; ‡Human Genetics Laboratory, GIGA-R Institute, University of Liege; §Independent Researcher; ‖Department of Human Genetics, CHU Liege, University of Liege; ¶Bioinformatics Unit, Department of Human Genetics, CHU Liege, University of Liege; #Department of Neonatology, CHU Liège, CHR de la Citadelle, University of Liège; **Department of Pediatrics, Division of Child Neurology, Reference Center for Neuromuscular Diseases, CHU Liege, University of Liege, Liege, Belgium; and ††Department of Pediatrics, MDUK Neuromuscular Center, University of Oxford, Oxford, UK.

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Reprints: Paulina Bartoszek, MD, Department of Ophthalmology, University Hospitals Saint-Luc, Institut Roi Albert II, UCLouvain, Avenue Hippocrate 10, 1200 Brussels Belgium (e-mail: paulina.bartoszek@saintluc.uclouvain.be).

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NOVEL APPROACH OF POPULATION SCREENING: BABY DETECT PROJECT

Newborn screening is a public health system test that identifies several congenital diseases for which timely treatment can preserve a child's health.[1] The classic Guthrie test has been used since the late 1960s in high-income countries and consists of a neonatal heel or arm prick to collect some drops of blood that are soaked into preprinted collection cards. Those blood samples can be tested for a variety of metabolic and genetic disorders. In Belgium, newborn screening tests include various diseases depending on the place where the child is born (Brussels-Wallonia vs. Flanders, Fig. 1). Since March 2021, after a 3-year pilot study,[2] spinal muscular atrophy—for which 3 effective treatments are now available[3]—has been included in Brussels-Wallonia. This test was added to the official screening program in December 2022 in Flanders.

Encouraged by the success of the study and the change it subsequently meant for 10 Belgian newborns treated before symptoms of the disease could appear, we set up a new 3-year pilot study, called Baby Detect Project, aiming to create a newborn screening test using targeted next-generation sequencing for all early-onset, treatable, and serious conditions. In its beginning, in September 2022, Baby Detect Study covered 126 diseases with 361 genes from 11 expert fields, including *RB1*, linked to retinoblastoma (Fig. 2). In September 2024, the panel was adapted to 405 genes, associated with 165 genetic

DISORDERS	WALLONIA	FLANDERS	Technique
INBORN ERRORS OF METABOLISM			
Phenylketonuria	X	X	LCMS
Maple Syrup Urine Disease (MSUD)	X	X	LCMS
Homocystinuria	X		LCMS
Tyrosinemia	X		LCMS
Propionic Acidemia	X	X	LCMS
Methylmalonic Acidemia	X	X	LCMS
Glutaric Acidemia type 1	X	X	LCMS
Isovaleric Acidemia	X	X	LCMS
Medium Chain Acyl-CoA DH deficiency (MCAD)	X	X	LCMS
Multiple Acyl-CoA DH deficiency (MADD)	X	X	LCMS
Very-Long Chain Acyl-CoA DH deficiency (VLCAD)	X		LCMS
Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency (LCHAD)	X		LCMS
Primary carnitine deficiency	X		LCMS
Galactosemia	X	X	Enzymatic
Biotinidase deficiency	X	X	Enzymatic
ENDOCRINAL DISORDERS			
Congenital Hypothyroidism	X	X	ELISA
Cystic Fibrosis	X	X	ELISA
Congenital Adrenal Hyperplasia	X	X	ELISA
OTHER			
SMA	X	X	qPCR

FIGURE 1. In Belgium, conventional newborn screening tests include various diseases depending on the place where the child is born (Wallonia vs. Flanders). These are tested with several different techniques. Spinal muscular atrophy (SMA) is highlighted as the last one to be added following the arrival of an effective treatment for this dreadful disease. ELISA indicates enzyme-linked immunosorbent assay; LCMS, liquid chromatography-mass spectrometry; qPCR, quantitative polymerase chain reaction. full color online

conditions, among which *RPE65* mutation associated retinal dystrophy, familial exudative vitreoretinopathy and congenital glaucoma.

By March 1, 2025, 6074 neonates had been tested. Baby Detect identified 114 positive neonates, of whom 19 were not covered by conventional newborn screening tests. All neonates received appropriate care. None of them had an *RB1* mutation.

The study is scheduled to end on May 31, 2025, with a subsequent ancillary study allowing long-term follow-up of children who test positive. The goal is to validate the scientific approach, check the genotype/phenotype correlation and determine how—in case of identification of any included mutation—the treatment, carried out early on, changes the child's prognosis.

It is to be noted that the study is very well received by parents, with over 91% of them agreeing to participate.[4]

RATIONALE FOR NEWBORN TESTING FOR *RB1* MUTATIONS

Retinoblastoma is the most common primary pediatric intraocular malignancy that develops in retinal cell precursors. Even though survival rates in developed countries exceed 95%,[5] the disease leads almost inevitably to more or less severe visual handicap. It is because at the young age where it typically presents (below 5 years), children are not able to report, or sometimes even notice, that something is going wrong with their sight, and the intraocular or extraocular signs such as leukocoria, strabismus, or cellulitis, that draw attention of the entourage, are synonymous to already advanced disease. As retinoblastoma destroys healthy retina, vision prognosis is primarily dependent on the delay in diagnosis and treatment, and secondly, on the tumor location (centrally located tumours giving more significant visual loss).

Most retinoblastomas—and all hereditary ones—are initiated by biallelic mutation of the retinoblastoma tumor suppressor gene, *RB1*, in developing retinal cells.[6] In case of a germline mutation, multifocal tumours develop early on, commonly before birth in at least one eye, second eye following in 90% of cases, with tumors detectable by the age of 6 months. Apart from familial cases, mean age at diagnosis in that group is 12 months, which means a delay

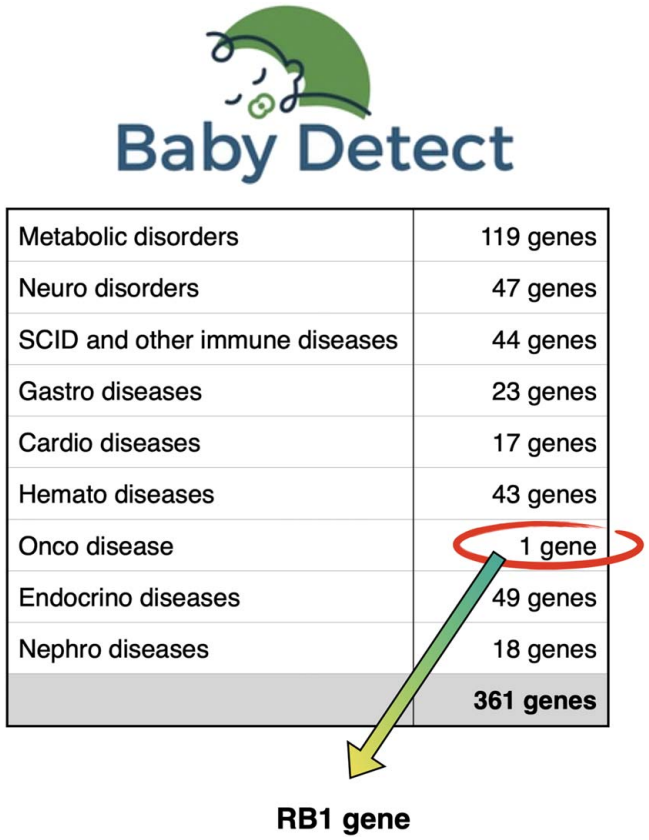

FIGURE 2. At its beginning (September 2022), Baby Detect's target panel covered 126 diseases with 361 genes from 11 expert fields, including gene *RB1*, linked to retinoblastoma. SCID indicates severe combined immunodeficiency. full color online

of 1 year in treatment, with most children having an advanced International Intraocular Retinoblastoma Classification[7] group D (intraocular diffuse disease with significant vitreous or subretinal seeding) or E (additional features indicating poor prognosis for eye salvage) in the worst eye and groupe B (intraretinal tumors centrally located or larger than 3 mm) or C (limited local disease with minimal subretinal or vitreous seeding) in the best eye.

Germline mutation concerns all bilateral retinoblastomas —40% of all cases—and up to 15% of unilateral retinoblastomas, which accounts for ~50% of all retinoblastoma patients and 100% of the most severe, bilateral cases.

However, in only 10% of cases is the mutation known due to a positive family history. If one of the parents is affected, there is a 50% risk of transmitting the causative mutation to the child (at each pregnancy). The newborn baby undergoes an ophthalmological examination promptly (immediate in the same sentence) after birth, leading to immediate treatment if any tumors are found. Those familial retinoblastomas are therefore detected and treated early on, in the neonatal period (first 4 weeks of life), which drastically changes eye and vision prognosis compared with nonfamilial cases. Indeed, in the neonatal retinoblastoma, as noted by Kivela and Hadjistilianou,[8] the worst scenario in the most affected eye is group C, whereas in the "better eye" the worst scenario is group B. Further on, the macula and good vision are likely to be spared in at least one eye. Children treated in the neonatal period have thus better vision outcomes, and less invasive therapy with less general anesthesia.

Because identification of *RB1* mutation has become a part of clinical management of patients with retinoblastoma for 2 decades now, the database of known causative mutations is robust. Therefore, testing for *RB1* mutation in a newborn screening test is of great interest.

INCIDENCE OF EXPECTED CASES, FALSE POSITIVE AND NEGATIVE VALUES, COSTS OF THE TEST

Retinoblastoma is a rare disease, occurring in ~1 out of every 16,000 to 18,000 live births in the global population.[9] In Belgium, over 100,000 babies are born every year.[10] We could expect around 6 new cases per year, 3 of them having an *RB1* germline mutation and thus detectable with newborn genetic screening. However, taking into consideration the known incidence of the diseases included in the panel[11] (https://www.rx-genes.com/about/) and available study results, we could expect a minimum of 1500 babies to test positive for any disease from the panel each year.

Obviously, while considering the usefulness of a test, not only the frequency but also the gravity of the disease should be considered, with the socioeconomic impact on the individual and their family. The example of spinal muscular atrophy, with around 12 new cases per year in Belgium, showed that newborn screening coupled with early treatment is cost-effective compared with late treatment following clinical diagnosis and is dominant when societal perspective, caregiver burden, and treatment based on parental preference are considered.[12]

It is important to stress that there are some ethical concerns related to the genetic nature of the screening. The test is not diagnostic, and the diagnosis needs to be confirmed. A baby with a positive result for any tested mutation has a high probability of having the disease, but could never develop it. This situation can be very stressful to parents who do not know what to expect while waiting for the definitive result.

Likewise, having a negative result (no mutation identified) does not mean that the child will never develop any of the concerned diseases. There could be new mutations not known (yet) to science and therefore not tested for. This is the reason why the test should regularly be revised to include all newly identified mutations.

Because only class 4 (likely pathogenic) and class 5 (pathogenic) variants of single-nucleotide polymorphisms are communicated, the Baby Detect test has a high positive predictive value of 98.2%. The negative predictive value exceeds 99.9%. So far, 2 falsely negative results were linked to mutations that had not been reported previously and consequently added to the variant database.

Providing parents with correct information is thus crucial.

The current screening test used in Belgium costs around 50 euros and is entirely covered by governmental agencies, Office de la Naissance et de l'Enfance in Brussels-Wallonia and Kind en Gezin in Flanders. It is offered to all parents at maternity wards; participation is voluntary.

The targeted next-generation sequencing test used in the Baby Detect Study is already commercially available and costs 650 euros. This price is linked to the project's limited scale and is expected to drop substantially with wide-scale implementation. Efforts are ongoing to obtain partial or full reimbursement of the test.

It is important to note that—just as it has been done in the study—the Baby Detect test should be run in addition to the standard Guthrie test with extra blood drops collected during the first 48 hours of life. Both tests are indeed complementary as biochemical screening has better sensitivity for inborn errors of metabolism and genomic approaches are currently not compatible with clinical onset of those disorders.[13]

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