

Genetic biomarkers for retinopathy of prematurity

Submission date 27/05/2022	Recruitment status No longer recruiting	<input type="checkbox"/> Prospectively registered <input type="checkbox"/> Protocol
Registration date 22/06/2022	Overall study status Completed	<input type="checkbox"/> Statistical analysis plan <input checked="" type="checkbox"/> Results
Last Edited 31/07/2025	Condition category Eye Diseases	<input type="checkbox"/> Individual participant data

Plain English summary of protocol

Background and study aims

Preterm infants, especially those who are born before the 32nd week of gestation or with a birth weight of less than 1500 g, are at risk of developing retinopathy of prematurity (ROP), a disease that affects the blood vessels of the retina (ocular fundus). Despite the gradual improvement in neonatal care and thus an increase in the survival of extremely premature babies, ROP remains the leading cause of childhood blindness and visual compromise in children. Premature infants develop ROP of different severities which mostly regress spontaneously and only a small number develop severe ROP. There are many factors involved in the disease and there is increasing evidence of the role of genetic factors in its development. The aims of this study are to determine the genetic factors that may contribute to the onset and progression of this disease, to identify circulating markers of the risk of developing ROP, and to analyze the influence of external factors in the development of ROP (such as nutrition, treatments, and environmental factors).

Who can participate?

Premature infants from eight Portuguese hospital centers, born before 32 weeks of gestational age or with a birth weight of less than 1500 g, and their mothers

What does the study involve?

This study will assess whether variants in genes (genetic polymorphisms) associated with vessel and nerve development, oxidative stress, and inflammation can influence the susceptibility to ROP and its progression. It will also evaluate the influence of epigenetic factors in preterm infants and their mothers through the study of heritable changes that do not involve alterations in the DNA sequence. The genetic study of the infants and mothers will be carried out using cheek swabs collected at 36 weeks of gestational age of the newborn or, if necessary, in the case of the newborn, with the surplus of blood from the first weeks of life. The sample of preterm infants will be subdivided into groups: infants who do not develop ROP will be the control group, and infants who develop ROP will be separated into groups according to the maximum stage of ROP reached. The distribution of the genetic polymorphisms studied will be compared between the infant groups that develop ROP and the group that does not develop ROP (control group). The study will also analyze and compare the importance of some blood markers in the early identification of preterm infants at higher risk of developing ROP. For this aim, the surplus

of blood from the routine laboratory tests of these infants in the first weeks of life is used. The data collected on nutritional aspects (e.g., breastfeeding versus preterm formula), infections (e.g., pneumonia) and treatments performed (e.g., antibiotics, blood transfusions) will be compared between the groups of infants to assess their possible role in the development of ROP.

What are the possible benefits and risks of participating?

There is no direct gain or benefit to the participants enrolled. No risks are expected. Knowledge of genetic and circulating markers associated with ROP may contribute in the future to help prevent complications associated with this disease.

Where is the study run from?

1. Genetics Laboratory and Institute of Environmental Health - ISAMB of the Lisbon School of Medicine (Portugal)
2. Bento da Rocha Cabral Scientific Research Institute (Portugal)
3. Cardiovascular Center of the University of Lisbon (Portugal)

When is the study starting and how long is it expected to run for?

April 2018 to August 2022

Who is funding the study?

1. Institute of Environmental Health - ISAMB of the Lisbon School of Medicine (Portugal)
2. Cardiovascular Center of the University of Lisbon (Portugal)
3. Bento da Rocha Cabral Scientific Research Institute (Portugal)
4. Portuguese Society of Ophthalmology (Portugal)
5. José de Mello Saúde (Portugal)
6. HeartGenetics, Genetics and Biotechnology SA (Portugal)

Who is the main contact?

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Contact information

Type(s)

Principal investigator

Contact name

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Additional identifiers

Study information

Scientific Title

Influence of genetic and epigenetic factors on susceptibility to retinopathy of prematurity and its progression

Acronym

GenE-ROP

Study objectives

1. Genetic polymorphisms (GP) with a minor allele frequency (MAF) greater than 10% in the general population, both in genes of pathways related to angiogenesis, neurodevelopment, oxidative stress, and inflammation in preterm newborns and in DNA methyltransferases of preterm newborns and their mothers, may influence susceptibility to retinopathy of prematurity (ROP) onset and progression.
2. Circulating biomarkers related to the studied GP may represent an effective way to identify premature newborns at greater risk of developing ROP and to know the phase of ROP in which the newborn is.
3. Co-factors of environmental, nutritional, therapeutic, and infectious origin, pre and postnatal, may influence ROP development.

Ethics approval required

Old ethics approval format

Ethics approval(s)

1. Approved 19/10/2018, Centro Hospitalar de Lisboa Norte (CHLN) and Centro Académico de Medicina de Lisboa (Academic Center of Medicine of Lisbon- CAML) Ethics Committee (Avenida Professor Egas Moniz, 1649-035, Lisboa, Portugal; +351 (0)217805405; comissaoeticacaml@chln.min-saude.pt), ref: 340/2018
2. Approved 11/01/2019, Hospital da Senhora da Oliveira Guimarães Ethics Committee (Rua dos Cutileiros 114, Creixomil, 4835-044 Guimarães, Portugal; +351 (0)253540250; centroacademico@hospitaldeguimaraes.min-saude.pt), ref: 01/19-CAC
3. Approved 21/01/2019, Centro Hospitalar Universitário de São João Ethics Committee (Alameda Prof. Hernâni Monteiro, 4200-319 Porto, Portugal; +351 (0)225512126; comissao.etica@chsj.min-saude.pt), ref: 20-19
4. Approved 12/12/2018, Hospital Professor Doutor Fernando Fonseca Ethics Committee (IC19, 276, 2720-276 Amadora, Portugal; +351 (0)214348200; uic@hff.min-saude.pt), ref: 70/2018
5. Approved 20/03/2019, Hospital de Braga Ethics Committee (Sete Fontes- São Victor, 4710-243 Braga, Portugal; +351 (0)253027249; daniela.esteves@ccabraga.org), ref: 15/2019
6. Approved 16/04/2019, Centro Hospitalar e Universitário de Coimbra Ethics Committee (Praceta Professor Mota Pinto, 3000-075 Coimbra, Portugal; +351 (0)239400477; UIDsubmissao@chuc.min-saude.pt), ref: CHUC-014-019

7. Approved 21/05/2019, Centro Hospitalar Universitário do Porto Ethics Committee (Largo do Professor Abel Salazar, 4099-001 Porto, Portugal; +351 (0)222077545; secretariado.cg.defi@chporto.min-saude.pt), ref: 2019.035 (031-DEFI/032-CE)

Study design

Multicenter prospective longitudinal case-control study based on a cohort

Primary study design

Observational

Study type(s)

Screening

Health condition(s) or problem(s) studied

Retinopathy of prematurity

Interventions

The preterm infants selected for the study, all of whom conform to the inclusion criteria and whose parents/ legal guardians have given their informed consent, come from eight Portuguese Care Units of Neonatology.

The genetic study of the infants will be carried out using:

1. The surplus of the blood that was collected and treated with EDTA for the routine laboratory parameters of these infants during the first 4 weeks of life

Or

2. From a buccal swab collected from the infant at 36 weeks of gestational age or at hospital discharge, the latter when collection at 36 weeks of gestational age is not possible.

The genetic study of the mothers will be carried out from the buccal swab collection.

A study of circulating biomarkers will be carried out with the surplus of plasma or serum once the routine laboratory parameters of these infants have been carried out in the first 4 weeks of life. Circulating biomarkers will be determined by a commercial ELISA kit.

A pen-and-paper self-administered questionnaire is being applied to the mothers to collect information on sociodemographic and behavioral aspects.

Clinical information on pathologies, treatments, nutritional aspects, and results of laboratory parameters is collected from the clinical process of the infant hospitalization, in the form of a questionnaire.

The ophthalmologist who performs the ROP screening of premature infants in the Neonatology Care Unit of each participant hospital is responsible for the ophthalmological records of each patient observed in the form of a questionnaire. Screening for ROP is performed by digital fundus retinography (RetCam) and/or binocular indirect ophthalmoscopy after the application of mydriatic eye drops according to standard practice in Portugal.

Intervention Type

Mixed

Primary outcome(s)

1. ROP staging (variable) is measured using the International Classification of ROP (I.C.R.O.P. -1984, revised in 2005). For ethical reasons and because it is an observational study with very premature infants, there are no medical tests made just for the study. The measure of this outcome comes from the ROP screening record performed by the ophthalmologists of the participating hospitals in accordance with the usual practice in Portugal. The first timepoint to

measure this variable depends on the gestational age at birth of the infant: for infants born before 27 weeks of gestational age, this variable is measured at 31 weeks of gestational age; for infants born after 27 weeks of gestational age, this variable is measured at 4 weeks of life. The following timepoints depend on the measurement result at the previous timepoint, as follows:

1. Stage 2 ROP in zone I and stage 3 ROP in zone II: between 5 and 7 days
2. Immature vascularization in zone I without ROP, stage 2 ROP in zone II and regressing ROP in zone I: between 1 and 2 weeks
3. Stage 1 ROP in zone 2 and regressing ROP in zone II: 2 weeks
4. Immature vascularization in zone II without ROP, Stage 1 or 2 in zone III and regressing ROP in zone III: between 2 and 3 weeks

The last timepoint to measure this variable (ROP staging) corresponds to the timepoint at which measurement result is complete retinal vascularization or complete remission of ROP.

ROP staging will allow the sample to be subdivided into groups. Infants who do not develop ROP will constitute the control group; infants who develop ROP will be separated into groups, according to the maximum stage of ROP reached and the need for treatment or not.

2. The following genetic polymorphisms of candidate genes in retinopathy of prematurity will be studied:

- 2.1. Angiotensinogen (rs699)
- 2.2. Angiotensin Converting Enzyme (ACE) (rs 1799752; rs 4291; insertion/deletion polymorphisms)
- 2.3. Angiotensin II type 1 receptor (AGTR1) (rs 5186; rs 427832)
- 2.4. Endothelial nitric oxide synthase (NOS3) (intron 4) (rs 1799983)
- 2.5. Endothelial nitric oxide synthase (NOS 2) (rs 229518; rs 9282801); rs 944722)
- 2.6. Catalase (CAT-262C/T) (rs1001179)
- 2.7. p22phox (CYBA C242T) (rs 4673)
- 2.8. Arginase (A2) (rs 3742879; rs 10483801; rs 17249437)
- 2.9. Haptoglobin (Hp) (rs 200299)
- 2.10. Superoxide dismutase (SOD1, 2 and 3): SOD1 (rs 17880135), SOD2 (rs 4880; rs 5746136) and SOD3 (rs 8192287; rs 2536512)
- 2.11. Heme-oxygenase 1 (HMOX-1) (-413 A/T) (rs 2071746; rs3074372)
- 2.12. Myeloperoxidase (MPO) (G463A) (rs 2333227)
- 2.13. Receptor for Advanced Glycation Endproducts (RAGE) (-429T/C) (rs 1800625)
- 2.14. Glutathione-peroxidase 1 (GPX1) (rs1050450)
- 2.15. Glutathione S transferases (GSTM1 and GSTT1)
- 2.16. Methylenetetrahydrofolate reductase (MTHFR C677T) (rs 1801133)
- 2.17. DNA methyltransferase 1 DNMT3B (rs 2424913)
- 2.18. Lysine-specific Demethylase 1 (LSD1) (rs 7548692)
- 2.19. Histone Deacetylase 3 (HDAC3 / SIRT3) (VNTR; G477T) (rs 11555236)
- 2.20. Vascular Endothelial Growth Factor A (VEGFA) -634 (CG) (rs 2010963)
- 2.21. Insulin-like Growth Factor 1 (IGF-1) (rs 9989002)
- 2.22. IGF-1 receptor (IGF-1 R) (rs 1319859; c.3174G>A)
- 2.23. Transforming growth factor beta 1 (TGFβ1) +869T/C(L10P); -509C/T (rs1800469)
- 2.24. Erythropoietin (EPO) (rs 507392)
- 2.25. Growth differentiation factor 6 (GDF6) (rs 6982567)
- 2.26. Brain Derived Neurotrophic Factor (BDNF) (rs 7934165)
- 2.27. Nerve Growth Factor (NGF) (rs6330)
- 2.28. Heparanase (HPSE) (rs4693608)
- 2.29. Tyrosine Hydroxylase (rs10770141)

The genetic study of all infants and mothers will be carried out from the buccal swab collection performed at 36 weeks of gestational age of the infant or at hospital discharge, the latter when collection at 36 weeks of gestational age is not possible. The buccal swab is collected from the mother at the same time as the infant. If the buccal swab collected from the infant is not enough

for the genetic study, for ethical reasons and because it is an observational study with very premature infants, the genetic study will be carried out with the surplus of blood collected and treated with EDTA during the first four weeks of life after routine laboratory parameters have been carried out.

Genotyping will be performed using a MicroChip DNA on a high-throughput platform using iPlex MassARRAY® technology from Agena Bioscience. The genotypes obtained are read by MALDI-TOF mass spectrometry. The different mass values of each generated PCR product are converted into genotype information. Genotyping data is analyzed using the EARTDECODE® software system from HeartGenetics.

The frequency of genotypes, the balance of their proportions, and the allelic frequency will be analyzed according to Hardy and Weinberg. The distribution of these polymorphisms will be used to compare the infant groups which develop ROP with the group that does not develop ROP (control group).

Key secondary outcome(s)

Circulating biomarkers:

1. Erythropoietin; insulin-like growth factor 1; brain-derived neurotrophic factor; regulated on activation, normal t cell expressed and secreted (RANTES); selectin; bilirubin. These variables will be measured using a commercial ELISA kit specific to each biomarker.
 2. High-sensitivity C-reactive protein (HS PCR). Determinations of this variable will be performed by immunoturbidimetry.
 3. Erythrogram and respective indices; leukogram and respective indices; thrombocytogram and respective indices. These variables will be measured using an automated hematology analyzer.
- As this is an observational study, all circulating biomarkers will be determined using the surplus of EDTA plasma or serum after routine laboratory parameters have been carried out in the infants during the first 4 weeks of life. For each of the circulating biomarkers, there are four timepoints, which correspond to the measurements performed during the first, second, third, and fourth week of life, respectively

Completion date

30/08/2022

Eligibility

Key inclusion criteria

Preterm infants and their mothers, with at least one of the following newborn characteristics:

1. Born before 32 weeks of gestational age
2. Born with a birth weight of less than 1500 g

Participant type(s)

Patient

Healthy volunteers allowed

No

Age group

Mixed

Sex

All

Total final enrolment

900

Key exclusion criteria**1. Infants:**

1.1. The existence of major congenital malformations

1.2. Ophthalmological pathologies, congenital or acquired (during the first 12 weeks of life) not related to ROP with the exception of conjunctivitis, keratitis and congenital obstruction of the tear-nasal canal

2. Mothers:

2.1. Diabetes

2.2. Gestation resulting from artificial fertilization

2.3. Insufficient clinical data

3. Legal guardians:

3.1. Absence of informed consent

It should be noted that when the mother is excluded, the infant can still form part of the study, provided informed consent is given.

Date of first enrolment

19/11/2018

Date of final enrolment

31/07/2021

Locations**Countries of recruitment**

Portugal

Study participating centre

Hospital de Santa Maria- Centro Hospitalar Universitário Lisboa Norte

Avenida Professor Egas Moniz

Lisboa

Portugal

1649-035

Study participating centre

Hospital Prof. Doutor Fernando da Fonseca

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Study participating centre

Maternidade Bissaya Barreto- Centro Hospitalar e Universitário de Coimbra
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Study participating centre
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Study participating centre
Centro Materno Infantil do Norte- Centro Hospitalar Universitário do Porto
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Study participating centre
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Alameda Prof. Hernâni Monteiro
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4200-319

Study participating centre
Hospital de Braga
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4710-243

Study participating centre
Hospital da Senhora da Oliveira
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4835-044

Sponsor information

Organisation

Institute of Environmental Health (ISAMB) of the Lisbon School of Medicine

Funder(s)

Funder type

Research organisation

Funder Name

Research Institute Bento da Rocha Cabral

Funder Name

Universidade de Lisboa

Alternative Name(s)

Universitas Olisiponensis, University of Lisbon, Technical University of Lisbon, ULisboa |
Universidade de Lisboa, University of Lisbon, Portugal, New University of Lisbon (Portugal),
ULisboa

Funding Body Type

Government organisation

Funding Body Subtype

Universities (academic only)

Location

Portugal

Funder Name

José de Mello Saúde

Alternative Name(s)

Funding Body Type

Private sector organisation

Funding Body Subtype

Other non-profit organizations

Location

Portugal

Funder Name

Heartgenetics, Genetics and Biotechnology SA

Funder Name

Portuguese Society of Ophthalmology

Results and Publications

Individual participant data (IPD) sharing plan

The datasets generated and/or analyzed during the present study are not expected to be made available individually, as they will be analyzed and presented grouped rather than individually. Access to the database is restricted to the principal investigator and another investigator of the team of the Genetics Laboratory and Institute of Environmental Health - ISAMB of the Lisbon School of Medicine. The identity and personal data of patients will never be disclosed, their confidentiality being ensured by encoding the identification elements of patients using an alphanumeric key. This alphanumeric key is registered in a separate Excel database, access to which is reserved exclusively for the principal investigator and, in each of the participating hospitals, the neonatologist and/or ophthalmologist responsible for collecting the clinical data necessary for the study in the respective hospital center.

IPD sharing plan summary

Not expected to be made available

Study outputs

Output type	Details	Date created	Date added	Peer reviewed?	Patient-facing?
Results article		02/05/2023	09/06/2023	Yes	No
Results article	Contribution of nerve growth factor (NGF) to ROP risk	22/01/2025	31/07/2025	Yes	No
Results article	Fetal hemoglobin as a predictive biomarker for ROP	06/01/2025	31/07/2025	Yes	No
Results article	Incidence, risk factors, and progression	24/09/2024	31/07/2025	Yes	No
Participant information sheet	Clinical research		13/06/2022	No	Yes
Participant information sheet	Susceptibility testing in clinical research		13/06/2022	No	Yes