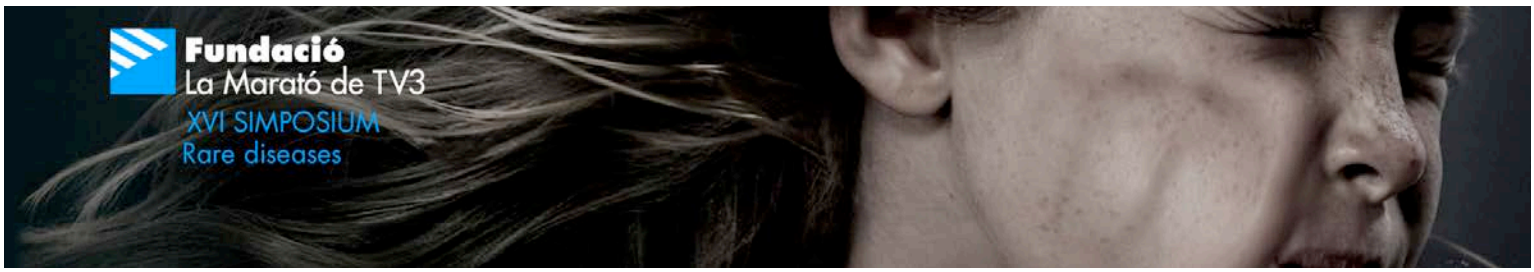

Creation of a Catalan network for diagnosis and clinical follow-up of the rare anaemias caused by major haemoglobinopathies

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1. Project Summary

CATGLOBIN "*Catalan Network for the diagnosis and monitoring of haemoglobinopathies and thalassaemias"* was funded by the Foundation "Marató TV3" during its "Rare Diseases" edition in 2009.

Haemoglobinopathies and thalassaemias are blood diseases belonging to the group of rare anaemias, affecting less than 5 per 10,000 inhabitants and their main clinical manifestation is anaemia. Some combinations of these blood diseases may cause what is known as sickle cell disease (SCD, which is an inherited disorder affecting haemoglobin, an essential component of red blood cells. It is estimated that one in every 4,494 newborns in Catalonia has the disease. In an infant with SCD, red blood cells can change shape, become rigid and obstruct small-diameter blood capillaries. These blockages can cause pain and damage to organs and tissues, serious infections and even death.

The main objective of CATGLOBIN is to create a network composed of a multidisciplinary team of paediatricians, haematologists, biologists and other specialists with the common goal of ensuring a correct diagnosis, prevention and clinical management of SCD, thalassaemia syndromes and other haemoglobinopathies associated with chronic anaemia. The achievement of this objective relies heavily on achieving the following specific objectives:

1. Promoting the development and standardization of biochemical and genetic methods for thalassaemia and haemoglobinopathy diagnosis, as well as updating clinical practice guidelines for adults and paediatric patients.

2. Establishing preventive programmes for severe syndromes of haemoglobin, such as detection of sickle cell anaemia or SCD and/or screening of couples at risk for SCD and thalassaemia syndromes, both recommended by the World Health Organization.

3. Creating a centralized report of these diseases as a tool for epidemiological monitoring and registration of clinical trials and cohort studies.

4. Studying the correlation between genotype and phenotype as the basis of the protocols for genetic counselling of severe syndromes of haemoglobin and identification of prognostic factors of the disease.

5. Disseminating knowledge of rare anaemias due to haemoglobinopathies in order to increase public awareness of these diseases among primary care practitioners, specialists, political bodies for health promotion, patients and the general public.

To perform these tasks, different coordination groups (WG) were set up with specific responsibilities, in which the different tasks were developed from a multidisciplinary point of view following this structure:

1. Group A (WGA): DIAGNOSIS AND PREVENTION

WGA-1 Biochemical and genetic diagnosis. Coordinator: Hospital Clínic

- Inventory of techniques available for the diagnosis of rare anaemias in centres that take part in the Marató team in Catalonia.
- Development of protocols for diagnosis and referral circuits samples.
- Development of biochemical and genetic techniques currently not available in any other location in Catalonia for the final diagnosis of any haemoglobinopathy present in our population.

WG A-2 Neonatal screening for haemoglobinopathies. Coordinator: Hospital Clínic

- Implementation of universal newborn screening for SCD.

WG A-3 genetic and prenatal diagnosis council. Coordinator: Hospital de Sant Pau

- Protocol to identify couples at risk and genetic counselling.
- Adaptation of genetic diagnostic techniques to prenatal diagnosis.

2. Group B (WG B): CLINICAL MONITORING AND TREATMENT

WG B-1 Paediatric clinical monitoring. Coordinator: Hospital de Sant Pau

- Review and update protocols for paediatric clinical follow.
- Inventory of services available for clinical monitoring centres in Catalonia.
- Protocols for referring patients to reference centres.

WG B-2 Adult clinical follow-up. Coordinator: Hospital Vall d'Hebron

- Review and update clinical monitoring protocols for adults.
- Recommendations for the multidisciplinary monitoring in adults: gynaecology, management of pregnancy in female patients, referral to genetic counselling of couples at risk.
- Inventory of services available for clinical monitoring centres in Catalonia.
- Protocols for referring patients to reference centres.

3. Group C (WG C): RECORDS

WG C-1 Epidemiological record. Coordinator: Hospital Clínic

- Anonymous correlated epidemiological report including all the diagnosed diseases, whether in heterozygous or homozygous state.

WG C-2 clinical registry. Coordinator: Hospital de Sant Pau

- Anonymous register correlated clinical diseases including only clinical follow-up: sickle cell anaemia, thalassaemia major and intermediate and other

haemoglobinopathies with clinical manifestations such as unstable or microcytosis of unknown origin.

4. D Group (WG D): CONTINUING EDUCATION

WG D-1 Training and educational activities. Coordinator: Hospital Clínic
- Organization of conferences, scientific meetings or courses for health professionals to provide information about the activities of the network, improve knowledge, and increase public awareness regarding the existence of rare anaemias.

WG D-2 Training and educational material. Coordinator: Hospital Clínic
- Development of training and educational materials for health professionals not experts in minority anaemias, and also patients and families so as to increase global awareness of minority anaemias.

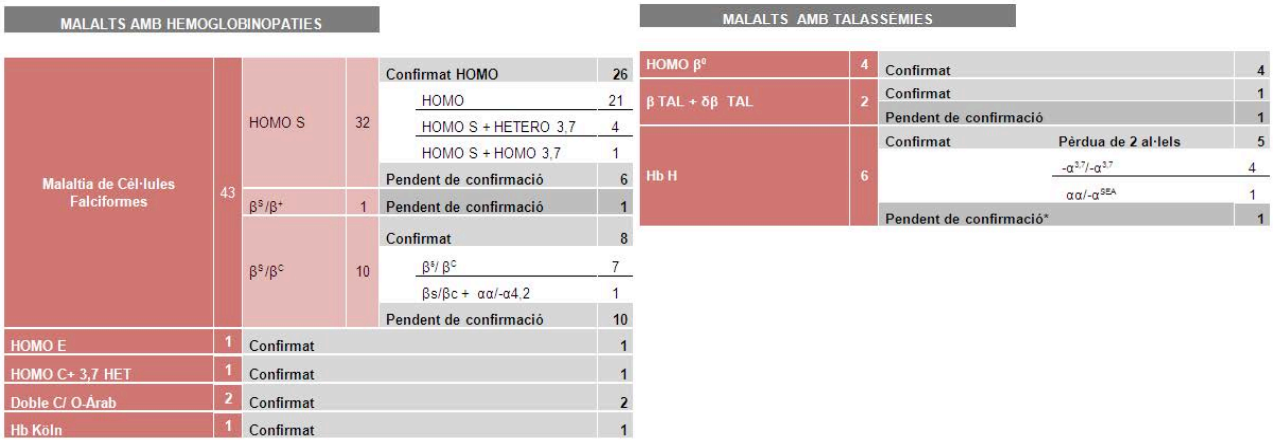
2. Results

Group A-diagnosis:

A1- Biochemical and clinical diagnosis: Results obtained in Catglobin
From the first half of January 2013 to November 5, 2014, 1,113 patients were registered in total. HPLC analysis confirmed the presence of thalassaemia or structural haemoglobinopathies in 705 of the 1,113 samples. These included 280 infants detected in neonatal screening and 361 families.

A total of 243 thalassaemia cases were identified (heterozygous or homozygous state), 368 cases of structural haemoglobinopathies (heterozygous or homozygous state) and 94 cases with both syndromes. Genetic characterization was performed on 491 of the 705 patients studied.

Out of the 1,113 patients studied, 60 were diagnosed and were divided into: 48 with haemoglobinopathies and one with thalassaemia. Haemoglobin S (Hb S) in homozygous state or sickle cell anaemia is the most common form of SCD, with 32 identified cases out of the 43 patients studied, representing 74.4%. **Tables 1 and 2**.



MALALTS AMB HEMOGLOBINOPATIES					
Malaltia de Cèl·lules Falciformes	43	HOMO S	32	Confirmat HOMO	26
				HOMO	21
				HOMO S + HETERO 3,7	4
				HOMO S + HOMO 3,7	1
				Pendent de confirmació	6
		β^S/β^+	1	Pendent de confirmació	1
		β^S/β^C	10	Confirmat	8
				β^S/β^C	7
				βs/βc + αα/-α4,2	1
				Pendent de confirmació	10
HOMO E	1	Confirmat			1
HOMO C+ 3,7 HET	1	Confirmat			1
Doble C/ O-Àrab	2	Confirmat			2
Hb Köln	1	Confirmat			1

MALALTS AMB TALASSÈMIES				
HOMO β^0	4	Confirmat		4
β TAL + δβ TAL	2	Confirmat		1
		Pendent de confirmació		1
Hb H	6	Confirmat	Pèrdua de 2 al·lels	5
			$-\alpha^{3.7}/-\alpha^{3.7}$	4
			$\alpha\alpha/-\alpha^{SEA}$	1
		Pendent de confirmació*		1

Tables 1 and 2. Totals of patients detected.

A2- Neonatal screening: Results obtained in the pilot test screening for sickle cell disease

From March 1, 2013 until February 28, 2014, we analysed a total of 26,971 babies.

Ten cases of SCD were detected, giving rise to a prevalence of 1/2,697 babies (0.037%) and 2 cases of ß-thalassaemia major with a prevalence of 1/1,386 babies (0.007%), 14 cases of suspected alpha- thalassaemia (Hb Barts) with a prevalence of 1/1,927 infants (0.052%), 2 cases of benign syndromes with a prevalence of 1/6,743 infants (0.015%) and 323 cases of heterozygote carriers of some variant of haemoglobin with a prevalence of 1/84 babies (1.198%).

Table 3. Results obtained in the SCD pilot test screening

FENOTIPS	Nº total	%	1 de cada
Malaltia cèl·lules falciformes			
FS - Malaltia cèl·lules falciformes	8	0,030	3.372
FSa - Malaltia cèl·lules falciformes	1	0,004	26.972
FSC - Malaltia de cèl·lules falciformes	1	0,004	26.972
Total- Malaltia cèl·lules falciformes	10	0,037	2.697
Síndromes talassèmics			
F - beta talassèmia major	2	0,007	13.486
FA - Barts* - alfa talassèmia	14	0,052	1.927
Total- síndromes talassèmics	16	0,059	1.686
Síndromes benignes			
FDA	2	0,007	13.486
FCA	2	0,007	13.486
Total síndromes benignes	4	0,015	6.743
Portadors heterozigots			
FAS - Tret falciforme	234	0,868	115
FAC	60	0,222	450
FAD	6	0,022	4.495
FAE	8	0,030	3.372
FAX beta	8	0,030	3.372
FAX alfa	7	0,026	3.853
Total- Portadors heterozigots	323	1,198	84

Furthermore, the familial studies of the babies carriers of Hb S, which have not all been completed, allowed the detection of four couples at risk of SCD (5% of total number of couples tested) and the diagnosis of a one-year sibling affected by SCD, who had not been diagnosed yet.

Table 4. Estimated prevalence of haemoglobinopathies in the neonatal population of Catalonia

POBLACIÓ NEONATAL TOTAL*	71975	
FENOTIP	INCIDÈNCIA ESTIMADA	1 DE CADA X
MALALTIA DE CÈL·LULES FALCIFORME	0,0256	3909
BETA TALASSÈMIA MAJOR	0,005	20289
ALFA TALASSÈMIA (PÈRDUA 2-3 AL·LELS)	0,041	2448
TRET FALCIFORME	0,693	144
PORTADOR HbC	0,184	544
PORTADOR HbD	0,017	5791
PORTADOR HbE	0,021	4665
PORTADOR HbX - BETA	0,025	3991
PORTADOR HbX - ALFA	0,020	4973
SÍNDROMES BENIGNES	0,011	9130

These results belong to the neonatal population of hospitals participating in the pilot study. This population shows a higher percentage of SCD than the global neonatal population of Catalonia. In order to extrapolate these results to the overall population in the same period of the study, five groups of patients were established according to the maternal origin of those babies with a haemoglobinopathy in the pilot study.

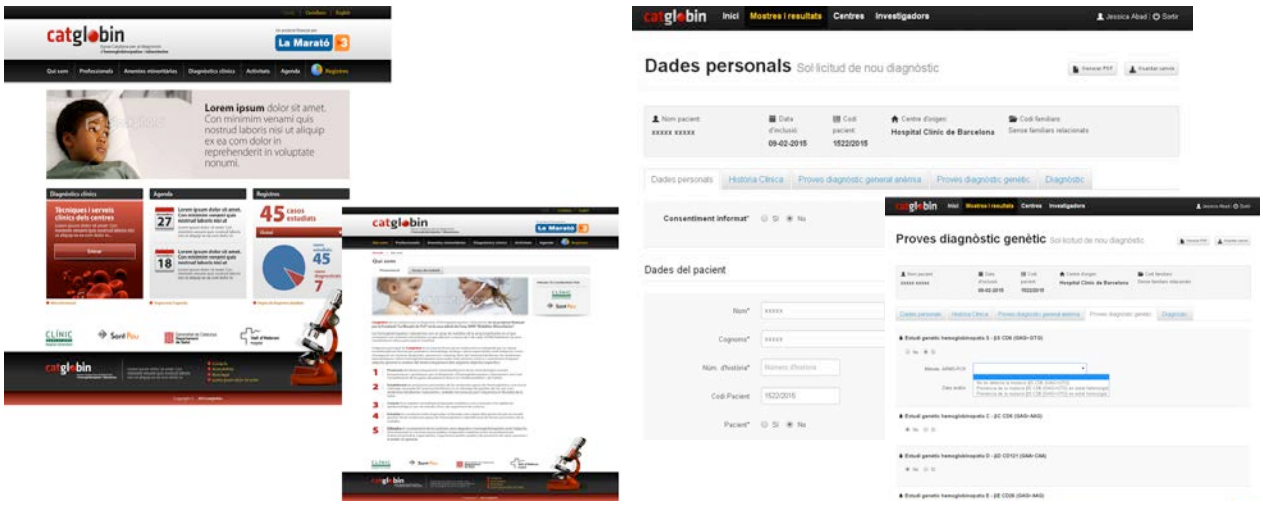
The prevalence was calculated in each of the groups. Thus the number of expected cases was extrapolated for each phenotype in the overall population of Catalonia at the same time as the pilot study, when there were a total of 71,975 newborns in Catalonia. The final prevalence estimated for the neonatal population of Catalonia is shown in **Table 4**.

Group C- Record:

C1- Epidemiological record and publication of results

This group was responsible for the design and development of the website project with the public environment, internal manager samples, epidemiological and clinical report. The website was published during the first half of January 2013:http://www.catglobin.cat/

Public part: Internal manager samples and patients



Group D-Education and training:

D1-Meetings and conferences

The following activities were organized in relation to the educational objectives:

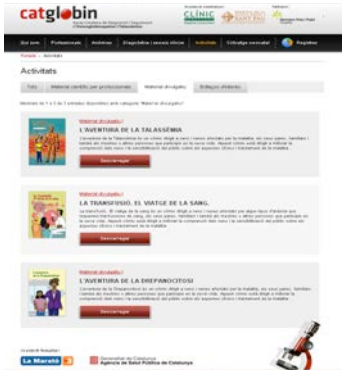
1st day, held on October 10, 2011: Diagnosis and prevention of haemoglobinopathies

2nd day, held on November 4, 2011: Clinical management of sickle cell anaemia

3rd day, held on December 1, 2011: Iron overload and chelating agents

4th day, held on January 12, 2012: Anaemias too difficult diagnose

D2 Educational material



Comics were published aimed at children affected by the disease, their parents, relatives, and teachers and other people involved in their lives. These comics are aimed at improving the understanding of children and public awareness of the clinical aspects and treatment of the disease:

http://www.catglobin.cat/activitats/cat/1/material-divulgatiu

D3-Diffusion in the media

In order to reach the general public and for publicity of rare anaemias, the network coordinator was interviewed on public television in Catalonia.

https://www.youtube.com/watch?v=dEJExRjAJG4

3. Relevance and possible clinical implications of the final results

Group A-diagnosis:

A1- Biochemical and clinical diagnosis: Results obtained in Catglobin

The **techniques developed** in molecular biology **have allowed the characterization of 100% of the mutations** responsible for the phenotype identified**.**

The network, consisting of a **multidisciplinary team**, allowed the analysis of genotype-phenotype association, the relationship of each mutation with haematological parameters and different fractions of Hb, and improved knowledge of the clinical expression and severity according to different genetic combinations.

This programme helped to promote **clinical practice guidelines** in adults and paediatric patients, which have been applied in 60 patients detected both in screening and in healthcare.

The group has been recognized by the Catalan government as an SGR group (support for research groups) **on rare anaemias**. Calling 2014

A2- Neonatal screening: Results obtained in the sickle cell disease pilot

The analytical methodology used in the screening is robust, and is adapted to the performance of a neonatal screening programme. The system **has full capacity to quantify haemoglobin F, A, S,** and other less common variants.

The molecular biology techniques developed made it possible to establish the risk of developing a severe haemoglobin syndrome, SCD or thalassaemia and improving the knowledge of genotype-phenotype correlation.

It has been estimated that the prevalence of SCD in **the neonatal population of Catalonia is 1 in every 3,909 babies (0.026%).** This prevalence places SCD as the second most prevalent disease currently included in the early detection neonatal programme (EDNP). This means that the annual number of expected cases of SCD and sickle cell trait is 18.3 babies and 499 babies, respectively.

The prevalence of both SCD and sickle cell trait **varies on the hospital**, showing a heterogeneous distribution of Hb S in the health area of Catalonia. This is **important for the selection of the reference centres** for clinical monitoring of patients once diagnosed.

Familial studies of children suffering from both SCD or having a haemoglobinopathy in heterozygous state **have allowed the detection of new cases in the same family. It has also been crucial in** the detection **of 4 couples at risk** leading to **proper genetic counselling and the possibility of offering prenatal diagnosis when necessary.**

The implementation of newborn screening for SCD in EDNP does **not require an additional sample**.

The Catalan Public Health Agency assessed the results of this pilot study and decided to include sickle cell disease in the Early Detection Neonatal Programme. This has been done since January 2015 in all newborns in Catalonia.

Group C- Record:

C1 -Epidemiological record and publication of results

The creation of Catglobin made it possible to centralise these diseases as a tool for monitoring epidemiological, clinical and cohort studies, responding to the request of the European Commission.

The website has made it possible for users to have knowledge of rare anaemias caused by haemoglobinopathies, a tool that increases public awareness of these diseases among primary care practitioners, specialists, public policy bodies for health promotion, patients and the general public.

Group D Education and training:

D1- Meetings and conferences

Conferences on rare anaemias, aimed both at health professionals who work with these diseases and at primary care professionals have been key to resolving questions of sickle cell disease and the clinical management of these patients.

4. Publications or communications derived from this research

X Annual meeting of "International conference on rare diseases and orphan drugs". 7-9 October 2014, Ede, The Netherlands: PILOT PROGRAM ON NEONATAL SCREENING FOR SICKLE CELL DISEASE IN CATALONIA -María del Mar Mañú Pereira1, José Luis Marín Soria2, Jessica Abad López1, Victoria Gutierrez1, Laura Olaya1, Joan Lluis Vives Corrons1, 1Unidad de Eritropatología. 2Laboratorio de Cribado neonatal. Hospital Clínic de Barcelona.

LVI CONGRESO NACIONAL SEHH - XXX CONGRESO NACIONAL SETH. 6-8 November 2014, Madrid, España: ESTUDIO COMPARATIVO ENTRE EL CRIBADO NEONATAL DE HEMOGLOBINOPATIAS SELECTIVO EN POBLACIÓN DE RIESGO FRENTE AL CRIBADO NEONATAL UNIVERSAL. EXPERIENCIA EN EL HOSPITAL PARC TAULI DE SABADELL DURANTE 1 AÑO.- L Muñoz, G Perea, M Melo*, J Badía*, J Guiu, J Obiols, MM Mañu**, JL Marín***, JLL Vives**. Laboratorio UDIAT, * Sevicio de Pediatria, Corporación Universitaria Parc Taulí, Sabadell; ** Laboratorio de Eritropatología, Centre de Diagnòstic biomèdic, *** Servicio de Bioquímica i Genética molecular, Hospital Clínic Barcelona.

VII Congreso Nacional del LABORATORIO CLÍNICO. Bilbao, 23 to 25 October 2013: PROGRAMA PILOTO DE CRIBADO NEONATAL DE LA ANEMIA FALCIFORME EN CATALUÑA.- Mañú Pereira M, Marín Soria JL, Abad López A, Vives Corrons JL.

VII Congreso Nacional del LABORATORIO CLÍNICO. Bilbao, 23 to 25 October 2013: EPIDEMIOLOGÍA MOLECULAR DE LOS SÍNDROMES FALCIFORMES Y TALASÉMICOS EN CATALUÑA**.-** Abad López J, Mañú Pereira M, Llaudet Planas, Vives Corrons JL