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PREVENTING PREMATURE CORONARY HEART DISEASE IN CATALONIA BY EXPANDING FAMILIAL HYPERCHOLESTEROLEMIA DIAGNOSIS

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1. Summary of the project

Familial hypercholesterolemia (FH) is the first monogenic conditioning of premature coronary heart disease (CHD). FH diagnosis is important because treatment greatly reduces cardiovascular morbidity and mortality in a cost-effective way. As pointed out by the European Society of Cardiology, FH is underdiagnosed and undertreated across Europe. According to the Catalan Health Service, 23% of the 15,000 expected FH cases in Catalonia have already been clinically diagnosed, but only 7% have been subjected to genetic testing. Moreover, there is an insufficient direct cascade genetic testing of mutation positive index cases. An inverse cascade is important as studying hypercholesterolaemic children can also lead to increased FH detection. At least 30% of the subjects with a clinical diagnosis of certain FH do not have identifiable mutations in the main FH genes, suggesting that some could have a polygenic FH.

Objectives

i) to increase the detection of FH patients by improving FH awareness among physicians taking care of patients with CHD, ii) to implement a direct and reverse cascade screening protocol addressed to early identification of FH, iii) to compare the FH detection rate resulting from objectives 1 and 2 with the current situation, iv) to set up a global FH detection programme in Catalonia according to the results and experience gained from the above objectives, involving the Catalan Lipid Net, and including the development of a specific FH website and genetic counselling to improve information to patients, families and health care workers, v) to define the clinical characteristics (lipid profile by NMR and subclinical arteriosclerosis) of these patients at diagnosis, vi) to test the recently proposed diagnosis of polygenic FH after genotyping of the main SNPs influencing LDL cholesterol, and vii) to study whether patients with familial combined hyperlipidaemia present a sizeable proportion of FH-causing mutations.

2. Main results

Increasing FH awareness

Joint protocols (lipidologist, cardiologist, family physicians and paediatricians) to detect FH candidates among patients suffering from precocious CHD, and adult or children with

high by cholesterol levels to implement FH diagnosis procedures have been written and clinically implemented.

Direct cascade screening

Direct cascade screening family studies (first degree relatives) of genetically diagnosed FH patients, with special attention to children has been implemented in our centre. Although the original design was focused on screening of FH relatives, we included 100 additional genetic diagnosis of putative index cases from the Catalan lipid clinic network. A total of 769 FH relatives were studied and results are reported in the Substudy 1 report About 464 (60°/) are positive increasing the suitability of FH relatives for genetic screening.

In our hospital we studied 94 offspring from 61 adults with definite FH diagnosis, either by genetic or clinically if parents had negative genetic test. So far, we have identified 68 new cases in children. Because of the high performance of the method, we decided to establish the protocol in our clinical practice.

Inverse cascade screening

To organize in our hospital the studies of parents of candidate children including genetic testing. Apply the same direct cascade protocol in confirmed FH families. Fifty-nine paediatricians, who were responsible for 63,616 children, were involved in the project. After 3,540 complete lipid profiles 122 children were sent to our lipid unit. 98 FH children plus 41 FH adults were detected (32 children and 35 relatives by inverse cascade). Because of the high performance of the method, we have established the protocol in our clinical practice.

Increasing the number of adult and children with diagnosed FH

Fourteen Units from the Lipids and Arteriosclerosis Units Net (XULA) in Catalonia were involved in the project. The number of new cases sent to genetic testing and registered to SCS FH register increased 1.5-fold.

From 2015 to 2019, 155 FH new patients diagnosed in our Unit were registered in the official SCS FH register.

From 2016 to 2019, 1,368 new patients were included in the SCS register, 816 of them with positive genetic testing. Because 298 of them are in the process of registration, the final figure of registered patients should be 1,666 new registers. Compared with the period from 2012-2015 (1,173 new diagnosis and registers) the diagnosis and register rate increased by 42%. The percentage of diagnosed children remains low (5,7%). The diagnosis is delayed in women with respect to men.

Establishing a programme

Lipid units from XULA to set-up the same protocols with the coordination of SCS.
As mentioned in the previous point, although 27 Units joined the project, finally 14 units have implemented the detection programme at least in part, mainly in the direct cascade screening part

Comprehensive FH phenotyping

Lipid profile by RNM in FH patients, IMT and arterial stiffness studies were performed in adult and child patients from our centre. Plasma lipoprotein NMR studies and new plasma biomarkers (IDOL, PCSK9, LDLR) were studied in 186 children. *Data are contained in two manuscripts already published.*
A full analysis of NMR data of adult FH patients including lipoprotein number and size and glycoproteins was performed in 320 FH adult patients, finishing in December 2019. The biochemical analysis period is finished and statistical studies are ongoing.

Implementation of lifestyle change programme

A specific programme has been prepared. Teaching materials are already available. A workshop programme has been performed. A total of 3 educational workshops on lifestyle education were carried out: 1 per child between 5-7 y/o; two per child from 5-12 y/o. Each workshop was organized in 6 sessions based on play and learn method focused on healthy lifestyle education. A paper with preliminary data has been published.

An international comparison is going on with the aim of assessing the impact of Mediterranean diet versus Nordic diet. In order to obtain this information a member of our group spent three month in Oslo University comparing these data.

Development of the new family hypercholesterolemia website

In 2019 we counted 1,171 visits to the website created, which was visited 3,601 times during the duration of the project. Several patients asked us questions about hyperlipidaemia, which were answered quickly. A total of 12 relatives of patients with familial hypercholesterolemia have contacted us through this website and they were genetically studied in our hospital. These are cases in which, for different reasons, it was difficult or impossible to obtain the biological sample in their usual health centre.

Genetic counselling

Demand was less than expected, given that the doctors of the different centres of the Xarxa d'Unitats de Lípids i Arteriosclerosi (XULA) carried it out. Biological samples (saliva, for greater convenience) from 12 relatives corresponding to 5 families directly in Sant Pau (apart from the usual healthcare procedure).

Previously, the necessary informed consents were explained to them, and how encrypted results would be sent that they should provide to their family doctor, paediatrician or dyslipidaemia specialist in their geographic area.

Sequencing the RLDL gene in 50 families with combined familial hyperlipidaemia

Only 1 patient of those studied with combined familial hyperlipidaemia had a mutation in the RLDL gene. These data are consistent with previous data indicating that mutations in RLDL are not a frequent cause of hypercholesterolemia in patients with familial combined hyperlipidaemia.

Collection of samples and genetic study of mutations of first-degree relatives

In the last year, a total of 227 samples of blood or saliva (230 in 2018) for genetic studies of relatives of index cases with familial hypercholesterolemia and a mutation identified in one of the following genes: RLDL, APOB, and PCSK9 (direct and reverse cascade). Therefore, and until now, a total of 769 samples of relatives have been processed. These partial results mean multiplying by 2.4 the number of family studies that we were conducted annually before the La Marató project (usually about 80 a year from XULA centres). We have therefore managed to study about 1/3 more of the 600 aimed at in the project. A total of 14 XULA hospital centres have sent samples and clinical information of these relatives allowing us to carry out this genetic study.

3. Relevance and future implications

This project is a clear example of translational research:

Our project was designed to evaluate the impact of screening protocols implementation in the detection of adult and child FH. An early detection must result in an early therapy and cardiovascular risk reduction. These actions cannot be isolated or restricted to a single centre but should be implemented by health systems through specific programmes.

Our project provides scientific evidence enough to change clinical practice leading to improved FH detection, therapy and prognosis. In our project we have tackled the following stages:

1. Implementation of FH detection protocols tailored to different specialities.
2. In-house coordination programmes between different hospital services and lipid units.
3. Coordination between different health care levels. Primary care and lipid units.
4. Coordination between lipid units of the Catalan Lipid Units Net (XULA).
5. Implementation of direct screening searching for index cases from suspected adults.
6. Implementation of reverse screenings searching for index cases from suspected children.
7. Implementation of the impact of new techniques based on NMR to obtain a more comprehensive knowledge of the clinical situation.
8. Implementation of lifestyle programmes particularly at early ages (children) in order to mitigate the effects of the disease at childhood.
9. Implementation of a dedicated website.

As a result of the project the following health care changes have been implemented

1. Different consensual protocols have been written to improve FH detection in cardiology, neurology, primary care physicians and primary care paediatricians. These protocols are active and followed at different rates. Coordination between hospital lipid units and primary care and paediatricians are active. These protocols are implemented and have been established beyond the duration of this project.

2. Up to 22 units form XULA were willing to collaborate in the La Marató TV3 programme. Finally, 8 units were actively implicated in the project. Algorithms addressed to genetic analyses indications and sample processing have been implemented in a coordinated way. This collaborative network will be maintained.

3. Protocols and algorithms to activate the search for FH first and second degree relatives (direct cascade screening) have been implemented and will remain as a standard of care in this population.

4. Protocols and algorithms to activate the FH detection in children have been implemented in the area of our own unit. This coordination will remain beyond the duration as a standard of care for early detection of FH.

5. It is possible in Catalonia to increase almost 2.5 times the rate of analysis of relatives of patients with familial hypercholesterolemia and an identified mutation, known as direct family waterfall genetic study, in the usual clinical context in which these studies are produced. This entails the possibility of treatment in a very cost-effective way.

6. In the context of this project a full set of protocols, and techniques, including group sessions to implement an early proper diet and overall healthy lifestyle, have been developed. This information has been made available to groups dealing with this disease through national and international networks.

There is no doubt that the results of our project have changed the clinical standards to detect and treat FH patients, also modifying the implementation of lifestyle changes and addressing the risk situation of these patients from early stages. This project will increase the yield of FH detection in Catalonia to that of leading European countries in this field.

Beyond translational aspects we have contributed by establishing new scientific evidence

1. Familial hypercholesterolemia is more frequent than expected. In the case of the homozygous form, with existing data it is at least two times more frequent than expected (1 / 450,000 people)

2. In familial hypocholesterolemia, as in familial hypercholesterolemia, it seems that there are forms of polygenic inheritance and not only monogenic.

3. There is an alteration in the functionality of HDL in patients with heterozygous familial hypercholesterolemia, specifically at the level of the reverse transport of cholesterol.

4. LDL also participate in the reverse transport of cholesterol, since they receive cholesterol of HDL in vivo, and channel its delivery to the liver through the LDL receptor. This route could be altered in patients with familial hypercholesterolemia and RLDL gene mutation, at least in homozygous forms.

5. NMR techniques will improve the clinical assessment of lipid disorders. In this project we have used these tests to analyse FH patients and evaluate its impact on cardiovascular risk detection. NMR assessed lipid profile has been conducted by the *Liposcale* test (patented by our group among others) and performed by a Universitat Rovira i Virgili spin-off (Biosfer Teslab). This project will help establishing the indication of this new way of studying lipid derangements. New clinical biomarkers have also been tested

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