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Assessing the improvements in the newborn screening strategy for cystic fibrosis in the Balearic Islands

Josep Miquel Bauça [a,*], Daniel Morell-Garcia [a], Magdalena Vila [a], Gerardo Pérez [a], Damián Heine-Suñer [b], Joan Figuerola [c]

[a] Servei d'Anàlisis Clíniques, Hospital Universitari Son Espases, Palma de Mallorca, Spain
[b] Servei de Genètica, Hospital Universitari Son Espases, Palma de Mallorca, Spain
[c] Servei de Pediatria, Hospital Universitari Son Espases, Palma de Mallorca, Spain

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ABSTRACT

Objectives: Newborn screening strategies for cystic fibrosis (CF) are run worldwide, and aim at the early detection of the disorder to significantly improve the quality of life. Elevated levels of immunoreactive trypsinogen (IRT) represent a high likelihood for the screened child to be affected with CF. However, the specificity of IRT is low. The objective of this study was to assess the screening program in the Balearic Islands during the past 14 years.

Design & methods: We evaluated all results of the screening program after 14 years, by considering all changes in the protocol and assessing the number of positive samples, the mutations detected, the number of sweat tests performed, the incidence of CF and the presence of false-negative cases.

Results: Despite a great variability among the different Balearic Islands, the global incidence of CF was 1:6059 for the 14 years assessed. The incidence in the smaller islands is about 5 times higher than in Majorca (1:2376 versus 1:10,613). After different changes in the protocol, an IRT cut-off value of 60 ng/mL was established. The two most common mutations are ΔF508 and G542X, in accordance with other geographical regions.

Conclusions: The changes in the protocol helped reduce the number of sweat tests performed without any increase in the false-negative rate.

Introduction

Newborn screening is an integrated program that aims at the systematic identification of inborn errors of metabolism, endocrine disorders and other diseases in the first days after birth. With a high sensitivity, it looks for potentially harmful disorders that are not apparent or evident, and its basic principle relies on the fact that early detection and treatment will lead to improved outcomes by reducing morbidity and mortality.

Such screening programs started in the United States in 1960s and expanded worldwide, currently representing an important public health initiative in many countries. Nevertheless, the range of screened pathologies may vary according to ethical, epidemiological, economic and political issues.

In the Balearic Islands, an archipelago with an overall population of 1,113,506 (Fig. 1), the newborn screening program started in 1979, including only two pathologies: phenylketonuria (PKU) and congenital hypothyroidism (CH). It was not until 1999 when cystic fibrosis (CF) was also introduced. Since then, many updates and changes have occurred.

CF is the most common genetic disorder in Europe and North America, having greater incidence in the Caucasian ethnicity [1,2]. With an autosomal recessive inheritance, it is reported to have an incidence of 1 in 2000–2500 live births. The main clinical features of CF are abnormally high sodium and chloride concentrations in sweat, insufficient digestive function, exocrine pancreatic insufficiency, and an increase of viscous secretions from mucous glands which lead to obstructive lung disease and infections. According to a study performed in Wisconsin, newborn screening for CF enables an immediate and accurate diagnosis before the onset of clinical symptoms [3,4].

Current CF screening approaches are based on the quantification of immunoreactive trypsinogen (IRT) on dried blood spots. Elevated levels of IRT represent a high likelihood for the screened child to be affected with CF. However, the specificity of IRT is low. Alternative screening methods are continuously suggested to improve the efficacy of the programs [5], for instance, by adding determinations to the protocol, such as the pancreatitis-associated protein (PAP) [6,7].

Since ethical aspects regarding benefits and risks are still under debate, we decided to perform an integral evaluation of 14 years of CF screening program, taking into consideration all the changes in the

* Corresponding author at: Ctra. Valldemossa 79, module J+1, 07010 Palma de Mallorca, Spain. Fax: +34 871 909706.
E-mail address: pepmiquel@gmail.com (J.M. Bauça).

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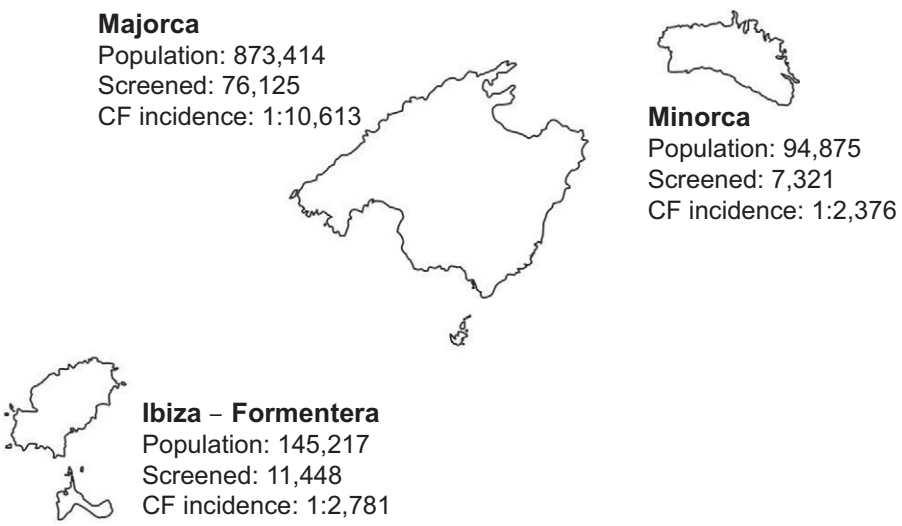

Fig. 1. Regional variations in the incidence of screened CF patients, from 2005 to 2013. (source for population: 2011 census).

protocol, assessing the benefits and flaws of each period, and checking all biochemical testing results, genetic analyses and outcomes.

Material and methods

Study population

The study population encompasses all the newborn in the Balearic Islands between January 2000 and October 2013. A total of 151,471 newborns were screened for CF. Although most of the inhabitants are of Caucasian origin, our population is relatively heterogeneous. This evaluation was performed according to the guidelines of the Investigation Ethics Board of the Balearic Islands.

Sample collection

Dried blood samples (on Whatman-903 filter paper) were obtained at all maternity units; both in public and private settings. Samples were drawn after 48 h of life before hospital discharge, and after two complete doses of either maternal or formula milk. Family data and informed consent were required for possible further genetic testing. Both demographical data registration and biochemical testing (IRT and sweat chloride) were performed in the Biochemistry Laboratory of Hospital Son Espases. Sample quality is controlled visually upon reception.

Further samples were required in cases of premature birth (<32 weeks of gestation), low weight at birth (<1500 g), blood transfusions, less than 2 complete milk ingestions or in cases of biochemical abnormal results.

Screening strategies

Different protocols were followed during the period, mainly due to improvements in the cut-off value for IRT. Performance of a sweat test (ST) and sequencing of the *CFTR* gene were carried out as confirmation (Fig. 2). The assessment of protocols was based on the number of positive samples obtained, the amount of samples with mutations detected, the number of ST performed, the incidence of CF and the presence of false-negative results.

Between September 1999 and October 2005, the protocol was based on two IRT quantifications, followed by mutation study and ST altogether (IRT/IRT/DNA + ST). From November 2005 until June 2006, one IRT determination was removed, and the cut-off value was followed at 60 ng/mL. From July 2006 to June 2008, IRT/DNA/ST, without a variation in the cut-off value. From July 2008 to January 2013, the protocol was the same, but the cut-off value was increased up to 70 ng/mL, by accepting the vendor kit recommendations and by performing a correlation study (data not shown). After February 2013, the cut-off value was lowered again down to 60 ng/mL (see Table 1), due to method reevaluation.

Current algorithm

IRT concentrations below 60 or 70 ng/mL were classified as negative for CF, and no additional testing was required. Results above the cut-off value were confirmed in the same sample before further analyses (DNA or ST). In case this 'apparently positive' result was confirmed, a filter paper aliquot was sent to the Clinical Genetics Laboratory together with the demographic data of the child.

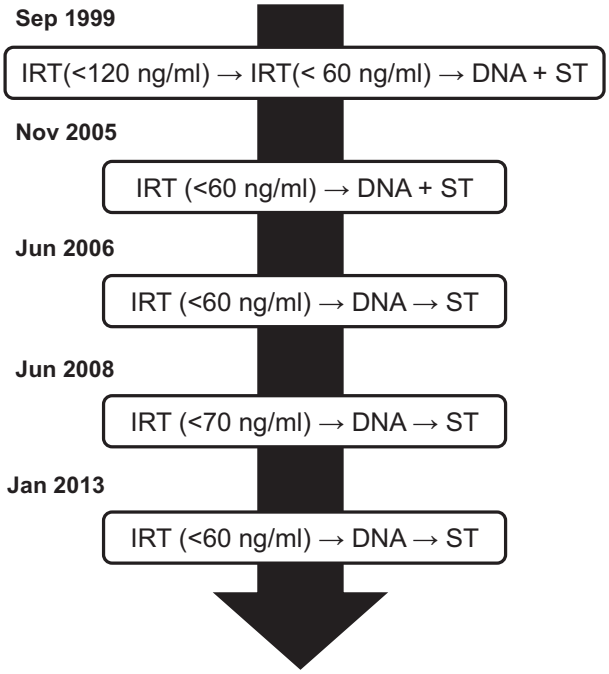

Fig. 2. Timeline with the different changes in the protocol.