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Clinical and molecular aspects of familial hypercholesterolemia in Ibero-American countries

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**Introduction**

Familial hypercholesterolemia (FH) is the most common monogenic disorder associated with premature coronary artery disease. Recently, due to advances in molecular diagnosis and pharmacologic treatment, there has been a renewed interest in identifying individuals with FH with intention to close the gap of care for this disease to prevent early cardiovascular disease and mortality. In the past years, many regional and international guidelines and consensus articles were published pointing out the importance of FH screening programs implementation aiming at early diagnosis and effective treatment of this high-risk population.

The Ibero-American community includes countries in Europe and America that speak Spanish and Portuguese and share a common genetic and cultural background with an estimated population of 640 million inhabitants (accessed at https://en.wikipedia.org/wiki/Ibero-America#Countries_and_population_in_Europe_and_the_Americas on July 29, 2106). Considering a prevalence of 1/300-500 for the heterozygous FH (HeFH) and 1/300,000-1,000,000 for the homozygous FH (HoFH), it is estimated that there are at least 2 to 2 millions of HeFH individuals and 600 to 1,800 HoFH in this region. Available information from different groups working on FH in those countries suggests that, with the exception of Spain, Portugal, and Uruguay, overall less than 1% has been diagnosed, being the detection rate higher in those countries with established screening programs.

The Ibero-American FH (IBAFH) network was constituted in Montevideo, Uruguay, in 2013 with the participation of physicians and biomedical researchers in FH from Argentina, Brazil, Chile, Mexico, Portugal, Spain, and Uruguay, thus representing almost 70% of the total population in the region, recently also Colombia joined the network. The main objectives of IBAFH network are to promote knowledge, education, and awareness about this disease for physicians, policy makers, patients, and general population. The IBAFH network also aims at improving FH diagnosis and treatment through the implementation of specific programs according to the differences in health systems in the region. It also intends to develop a common registry about FH in Ibero-America. Finally, it aims to reduce the high cardiovascular disease burden of this population.

The objective of this article is to describe the state of the art in current clinical and molecular FH diagnosis as well as efforts developed to reduce the cardiovascular disease burden caused by FH in IBAFH network country members.

**Material and methods**

This study describes the clinical and molecular epidemiology of FH in Argentina, Brazil, Chile, Mexico, Portugal, Spain, and Uruguay. Information about cascade screening programs, molecular diagnosis and the availability of local FH guidelines, LDL apheresis, and FH patient organizations was collected from country specific members as well as from publications in full papers in the English language when available. Since there is a common ancestry relation among member countries, the most frequent and common mutations found were reported.

**Results**

**Epidemiologic and cascade screening aspects**

Approximately, 27,400 FH patients have been identified by both clinical and molecular diagnoses in the 7 founder countries that constitute the IBAFH network. The data from
Colombia are still too incipient at the moment and were not reported. Table 1 shows the conservative estimation (1 of 500) for HeFH, number of identified individuals, number of identified children and adolescents, prevalence of cardiovascular disease as well use of molecular testing. The prevalence of cardiovascular disease in diagnosed patients varies from 10\% to 42\% depending on the country. The situation of FH diagnosis, treatment, and cascade screening programs in region countries is highly variable. Only in 4 countries (Brazil, Spain, Uruguay, and Portugal), there are established systematic programs for detection of the disorder. In Brazil, the approach is regional and performed in the State of Sao Paulo (Hipercol Brasil program accessed at http://hipercolesterolemia.com.br) since 2010. The program consists mainly of a genetic cascade screening approach and until the first half of 2016, 1260 individuals have been identified so far by molecular diagnosis. The Brazilian Society of Cardiology published its FH guidelines in 2012, and since 2014, there is an FH patient organization in Brazil (Associação Hipercolesterolemia Familiar [AHF]–www.ahfcolesterol.org).

In Spain, there are some local programs developed by regional governments in collaboration with the Spanish Familial Hypercholesterolemia Foundation, as well as a nationwide program developed by the Foundation in collaboration with lipid clinics and primary care physicians for cascade screening for FH in the framework of a translational research project known as Spanish Familial Hypercholesterolemia Study. Around 25,000 FH individuals have been identified in Spain by either molecular or clinical diagnoses. Therefore, it is estimated that 25\% of the Spanish FH population has been diagnosed. Spain has local specific guidelines for FH identification and treatment. In Spain, the Foundation also represents the rights of FH patients and chronic treatment for FH is available at no cost to patients including LDL apheresis (https://www.colesterolfamiliar.org).

In Uruguay, Genes y Colesterol (GENYCO) the National Program for Early Detection and Treatment of FH is promoted and supported by the Honorary Commission for Cardiovascular Health (accessed at http://cardiosalud.org/informacion-para-equipodesealud/genetica/genyco Web page in Spanish). It was created in 2012 (Law 18,996, item 12, article 207) by a regulatory decree (357/013) approved by the Ministry of Health in November 2013. GENYCO is a centralized national registry providing access to genetic testing and cascade screening. It coordinates the health-caring process for FH patients and their affected family members. Both public and private medical institutions identify suspected index cases using the Dutch Lipid Clinic Network criteria by a web-based software, and clinical information and a saliva sample are sent to the GENYCO coordinating unit for molecular diagnosis and cascade screening when indicated. The program also guarantees free access to standard lipid-lowering therapies.

Portugal does not have a governmental program for FH detection; however, in 1999, the National Health Institute Dr.

### Table 1

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of expected FH patients*</th>
<th>Number of index cases †</th>
<th>Number of positive cases ‡</th>
<th>Cardiovascular disease (%)</th>
<th>Total genetic identification rate (%)</th>
<th>Number of different variants found</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>100,000</td>
<td>3901</td>
<td>8337 (445)</td>
<td>13</td>
<td>8.3</td>
<td>436</td>
<td>#10, 21 and unpublished data</td>
</tr>
<tr>
<td>Portugal</td>
<td>20,600</td>
<td>832 (282)</td>
<td>786 (279)</td>
<td>13</td>
<td>3.82</td>
<td>258</td>
<td>#12 and unpublished data</td>
</tr>
<tr>
<td>Uruguay</td>
<td>6600</td>
<td>76 (36)</td>
<td>170 (25)</td>
<td>36</td>
<td>2.5</td>
<td>101</td>
<td>#11 and unpublished data</td>
</tr>
<tr>
<td>Brazil</td>
<td>400,000</td>
<td>339 (20)</td>
<td>1258 (147)</td>
<td>23.2</td>
<td>0.27</td>
<td>101</td>
<td>#14, 15, and unpublished data</td>
</tr>
<tr>
<td>Mexico</td>
<td>235,000</td>
<td>118 (74)</td>
<td>315 (37)</td>
<td>22.8</td>
<td>0.13</td>
<td>13</td>
<td>#14, 15, and unpublished data</td>
</tr>
<tr>
<td>Chile</td>
<td>34,000</td>
<td>35 (35)</td>
<td>42 (15)</td>
<td>20</td>
<td>0.05</td>
<td>12</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>Argentina</td>
<td>98,000</td>
<td>51</td>
<td>51 (17)</td>
<td>30</td>
<td>0.05</td>
<td>20</td>
<td>Unpublished data</td>
</tr>
<tr>
<td>Colombia</td>
<td>98,000</td>
<td>20</td>
<td>20 (20)</td>
<td>10</td>
<td>0.01</td>
<td>20</td>
<td>Unpublished data</td>
</tr>
</tbody>
</table>

FH, familial hypercholesterolemia; IBAFH, Ibero American FH.

*Based on 1/500 prevalence.
† Total number of clinical FH with complete molecular study (index cases), in brackets the number of children mutation positive.
‡ Total number of index case and relative’s mutation positive, in brackets the number of children mutation positive.
Ricardo Jorge (INSA) started the Portuguese FH study with the main objective to know the real prevalence of the disorder and to determine the genetic cause of the dyslipidemia in these patients. The Portuguese FH Study is a national study to which more than 800 clinical FH index patients and more than 2000 relatives have been referred. Since 2012, Portugal has an FH patient organization (http://fhportugal.pt). A governmental FH registry is being developed.

In Mexico, it is estimated that there are more than 200,000 affected FH individuals; the great majority have not been diagnosed. Although there is no national program for FH detection and management, some regional studies have permitted the molecular analysis in 123 index cases finding a functional mutation in 64 of them. The cascade screening performed from these 64 cases, permitted to analyze 423 relatives, confirming the molecular diagnosis in 251 subjects. There are neither FH patient organizations nor specific FH medical guidelines in Mexico.

In Argentina, there are 60 patients detected with FH so far: 33 index cases (24 with genetic confirmation) from different cities of the country and 27 relatives. The first Argentinian FH registry (Da Vinci registry) was launched in 2015. This registry is a collaborative research between the government (Department of Health from Mar del Plata City) and 2 universities (the Laboratory of Lipid and Atherosclerosis from the University of Buenos Aires and FASTA University School of Medicine in Mar del Plata). Preliminary results of this study suggest a prevalence of 1/186 based on the Dutch Lipid Clinics Network Criteria (personal communication Dr Corral). No FH patient organization is active in Argentina until now; however, there is 1 FH practice guideline from the Argentinian Lipid Society.

In Chile, there are 30 cases diagnosed using molecular testing in collaboration with the Spanish FH Foundation and the Portuguese FH Study. Recently, the Chilean Ministry of Health has developed in collaboration with local specialists the guidelines for prevention of cardiovascular disease that include FH on its topics.

Within the IBAFH network countries, LDL apheresis is available only in Portugal and Spain.

**Molecular characterization of FH patients in the IBAFH network**

Despite a great heterogeneity among the screening programs, all countries included in this network have started FH molecular studies. Countries such as Spain, Portugal, and Brazil have a considerable number of patients with a complete genetic characterization (>700 patients). The remaining Latin American countries are still developing strategies for these studies implementation. The highest molecular identification rate is seen in Spain, 8.3%, followed by Portugal, 3.8%, and Uruguay with 2.5% of the population. In the rest of Ibero-American countries, the identification of FH patients is still less than 1%.

Spain was the only country in IBAFH network that had regional governmental approval for the genetic identification of FH patients, but Uruguay has recently approved a law for patient identification. These are the only 2 countries with a governmental involvement in FH identification.

Figure 1 shows the most common mutations among the network countries based on the 10 most common mutations of FH patients. In all countries, low density lipoprotein receptor (LDLR) mutations are the most common cause of FH. All countries have identified patients with the APOB3527 mutation except Chile. Portugal has the highest number of different functional APOB mutations due to having introduced recently the study of whole APOB gene in their genetic diagnosis. Proprotein convertase subtilisin kexin type 9 (PCSK9) mutations were found only in Spain and Portugal.

In the 3 countries with the highest numbers of identified FH patients (Spain, Portugal, and Brazil), 10 to 15 variants are responsible for 30% to 47% of all FH cases. In Spain, the 10 most common mutations represent 30.6% of total FH cases. The frequency of APOB 3527 is 3.3%, and for all APOB mutations, the frequency is 3.5%. PCSK9 mutations have been identified only in 3 families in Spain, presenting with the p.Asp374Tyr first described in UK patients.

In Portugal, the 10 most common mutations represent 46.64% of the total FH cases. The frequency of p.Arg527Gln is 3.56% and of all APOB mutations is 5.14%. Two unrelated patients (and 1 relative) were found to have the same PCSK9 mutation, the p.Asp374His, only reported in Portugal so far. Recently, the first PCSK9 homozygous patient was described in Portugal with 2 functional mutations, and 4 relatives were found to have one of these mutations.

In Brazil, the 15 most common mutations represent 47.2% of total FH cases. The frequency of APOB3527 is 0.8% and of all APOB mutations is 1.6%. No mutations in PCSK9 were found. The most frequent mutation was of Lebanese origin, the p.Cys681* in exon 14 of the LDLR (8.5%). This mutation also was also the most frequent found in Argentina, being present in 18% of index cases (Virginia Baiares personal communication). The remaining mutations present in more than 1 index case from Argentina were also found in Spain.

In Mexico, most mutations were located in the LDLR gene (95%); the 5 most common were c.1055G>A, c.1090T>C, c.682G>A, c.2271delT, and c.338insG; the latter 2 reported only in Mexicans.

Chile started recently the molecular study of their patients having identified 30 patients with a putative causing mutation and 2 true homozygotes.

Figure 1 shows that Spain and Portugal share 5 of the 10 most common mutations in each country (4 in LDLR and the APOB3527) and have a very similar prevalence of the APOB3527 mutation. Spain and Spanish-speaking Latin
American countries share 6 of the most common LDLR mutations and the APOB3527. Unexpectedly, Portugal and Brazil only share one of the most common mutations, but only a small percentage of Brazilian patients have been identified so far so, most probably, Portugal and Brazil will share more FH alleles.

Due to historical relations among Ibero-American countries, an overlap of common FH mutations was expected, in fact, in the group of the most common mutations in each country, there were 11 mutations that have been identified in at least 2 countries (Fig. 1). There are some exclusive mutations, for example, the alteration [c.313+1G>C; c.274C>G(p.Gln92Glu)] that has only been described in Spain and Chile, and the promoter alteration c.-135C>G only reported in Ibero-America and USA, so to the best of our knowledge, these were not encountered so far in any of the other European, Asian, or African countries (unpublished data).

Table 2 shows the number of identified true homozygotes and compound heterozygote individuals. This number is also largely variable within Ibero-America countries, varying from 5% in Argentina to 100% in Portugal when a conservative prevalence of one in a million for homozygotes (true homozygote and compound heterozygotes) was considered.

**Figure 1** Most common mutations in IBAFH network (in the 7 countries that presently form the network), based on the 10 most common mutations in each country. Only mutations shared by at least by 2 countries are represented. Between brackets are the other countries where these alterations have been described. Data from Colombia are still very incipient. APOB, apolipoprotein B gene; IBFH, Ibero-American FH; LDLR, low density lipoprotein receptor gene.

**Discussion**

This study presents the available clinical and molecular aspects of FH in the Ibero-American region. The region shares common FH mutations, especially among Spanish-speaking countries and gaps in diagnosis and care of FH individuals.

With the exception of Spain where approximately 25% of possible FH individuals were diagnosed, so far, there is a tremendous gap in FH diagnosis and treatment in Ibero-America. This is exemplified by the elevated prevalence of previous cardiovascular disease in identified subjects of participating countries except for Spain due to its greater rate of early detection and possible earlier treatment onset in comparison with the other countries. Two other indicators of the diagnostic gap are the small number of FH children and homozygous individuals identified in most Ibero-American countries, a fact showing the absence of adequate cascade screening programs. Indeed, only Portugal and Spain have identified most of their individuals with a HoFH phenotype. This diagnosis and treatment gap can even be even greater if one considers a less conservative prevalence of the disease, 1/217 and 1/300,000 individuals as has been suggested by Benn et al. and Sjouke et al., respectively, for heterozygous and HoFH instead of the classical 1/500 and 1/1,000,000 that were used in this study.
Many hurdles for an adequate FH diagnosis and treatment in IBAFH network member countries were identified; however, the most important was the absence of organized cascade screening programs with national span. For instance, even considering the potential of the Hipercol Brasil program that could reach a population of 40 million people living only in the state of Sao Paulo, the rest of the Brazilian population approximately 160 million people would not be adequately covered. In addition, the lack of government funding for these programs in most member countries with the exception of Spain and Uruguay limits diagnostic efforts. Indeed, despite all member countries have collected information regarding molecular characteristics of affected individuals, only Spain and Uruguay have available government funding for molecular diagnosis. This fact endangers the potential for cascade screening efficacy because a positive molecular diagnosis has been previously shown to be cost-effective in identifying other FH individuals. Furthermore, the absence of FH screening and treatment guidelines in some countries, and the nonavailability of LDL apheresis in Latin America certainly impede the identification and adequate treatment of the more severe FH forms.

Despite limitations on molecular diagnosis availability, in this study, it was possible to show a snapshot of FH causing mutations in the region. More than 90% of FH causing mutations were found in the \textit{LDLR}, also 3.5% of individuals in Spain, and 5% of the Portuguese presented mutations in the \textit{APOB} gene while less than 1% presented mutations in the \textit{PCSK9} gene. The latter however were found only in Europe. The lack of \textit{PCSK9} mutations in Latin America could be due to the relative low number of patients genetically identified so far or it could be region specific, therefore, further studies are still necessary.

In Brazil, Portugal, and Spain, 10 to 15 mutations represented between 30% and 47% of all identified FH patients. Of interest, Spain and Portugal shared five of the 10 most common mutations, and most frequent encountered mutations were also shared among Spain and Spanish-speaking Latin America countries. Considering the historical links between Brazil and Portugal, one would have expected a greater sharing of common mutations between these 2 nations. However, one should consider the still small number of identified Brazilian FH individuals, in addition, despite the fact that Brazil received colonization and immigration by Portuguese individuals, it also had immigration waves from Sub-Saharan Africa (forced), Spain, Italy, Syria, Lebanon, Germany, and Japan as well as invasions by the Dutch and the French during colonial times. Therefore, further studies are necessary to better comprehend mutation spectrum in Brazil to compare it with other countries.

Currently, there is a renewed interest in identifying FH individuals not only because of the importance of this disease as a cause of early cardiovascular disease and death but also due to development of new medications to treat it. The IBAFH network aims at reducing the gap on awareness and treatment of FH as well as to stimulate research about this disease in the region. For that, so far, 4 annual FH symposia have been done not only with the purpose of medical education but also to stimulate cascade screening, to discuss local health policies for FH care, and to stimulate FH patients to start organizations to fight for their rights to adequate diagnosis and treatment. The IBAFH network activities can be reached in the Web site http://redhipercolesterolemiasfamiliar.net/sitio/(in Spanish). The network aims also at performing a registry about FH individuals to better characterize this disease in the region and also intends to invite other region countries to become members. Finally, country members also collaborate in other international initiatives to better characterize FH.

### Table 2

<table>
<thead>
<tr>
<th>Country</th>
<th>True homozygotes</th>
<th>Compound homozygotes</th>
<th>Total</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spain</td>
<td>27</td>
<td>4</td>
<td>34</td>
<td>#21 and <a href="http://safeheart.colesterolfamiliar.org/">http://safeheart.colesterolfamiliar.org/</a></td>
</tr>
<tr>
<td>Portugal</td>
<td>3</td>
<td>7</td>
<td>10</td>
<td>#12 and unpublished data</td>
</tr>
<tr>
<td>Brazil</td>
<td>15</td>
<td>6</td>
<td>21</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Uruguay</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Argentina</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Mexico</td>
<td>9</td>
<td>4</td>
<td>13</td>
<td>#15 and unpublished data</td>
</tr>
<tr>
<td>Chile</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>unpublished data</td>
</tr>
<tr>
<td>Colombia</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

FH, familial hypercholesterolemia; IBAFH, Ibero-American FH.

In conclusion, IBAFH network countries share common molecular causes and gaps in FH diagnosis and care. The IBAFH network aims at reducing this gap and increase identification and treatment of affected individuals to reduce the cardiovascular disease burden caused by FH in the region.

### Conclusions

In conclusion, IBAFH network countries share common molecular causes and gaps in FH diagnosis and care. The IBAFH network aims at reducing this gap and increase identification and treatment of affected individuals to reduce the cardiovascular disease burden caused by FH in the region.

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**References**


