Short Report

Balearic archipelago: three islands, three beta-thalassemia population patterns


The mutation spectrum of 175 β-thalassemia (β-thal) carriers, identified in pilot carrier screening on 22,713 individuals from Balearic Islands (Spain), is reported. The β0 CD39 (C>T) mutation is the most frequent (61.1%), followed by β+ IVS-I-110 (G>A) (12.0%), β+ IVS-I-6 (T>C) and β0 IVS-1-1 (G>A) (3.4% both) and eight other rare mutations (2.9–0.6%); with a distinct prevalence and distribution between islands. Minorca shows the highest prevalence in Iberian populations, with a single mutation, CD39 (C>T), present in most β-thal carriers. Ibiza is the only Western Mediterranean population where the most frequent β-thal mutation is IVS-I-110 (G>A). These results can be explained by a combination of historical–demographic characteristics together with evolutionary forces such as founder effect, genetic drift and probably selection by malaria. Knowledge of the mutational spectrum in the Balearic Islands will enable to optimize mutation detection strategy for genetic diagnosis of β-thal in these islands.

Conflict of interest

The authors declare no conflict of interest.

β-thalassemia (β-thal) is one of the most widespread monogenic diseases; caused by genetic mutations that either abolish (β0-thal) or reduce (β+-thal) the synthesis of β-globin chains, resulting in an imbalanced production of α and non-α chains (1) which triggers variable degrees of anaemia. Heterozygous individuals for a β-thal allele (β-thal trait) have mild microcytic anaemia. In homozygous or compound heterozygous patients, the phenotype generally depends on the severity of the β-thal alleles involved and environmental and genetic modifiers (1, 2).

β-thal is highly prevalent in Mediterranean countries and also in Southern Asia, India, Africa, Central America and the Middle East; whereby knowledge of the molecular pattern of β-thal is essential to manage these diseases in preventive programmes (1, 3).

About 200 β-thal mutations have been described. In Spain, studies of β-thal mutations in patients
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from different regions indicate the frequency of different alleles varies markedly between populations (reviewed in Ref. 4), although four mutations [(CD39 C>T), IVS-I-110 (G>A), IVS-I-6 (T>C), IVS-I-1 (G>A)] are the most frequent, as in other Mediterranean countries. Recent migratory flows from non-Mediterranean geographical areas have markedly increased β-thal mutation heterogeneity (3), highlighting the need for new regional studies.

Different people, especially Romans (third century BC) and Catalans (early thirteenth century), contributed to the genetic pool of the current Balearic population (Majorca, Minorca and Ibiza islands, in Western Mediterranean Sea), but no remarkable contribution of foreign genes was received until recently because of immigration. Genetic studies (5, 6) show differences between the three islands and the Ibizan population, probably because of the Phoenician—Carthaginian origin of the first settlers and to genetic drift.

The purpose of this study was to determine the incidence and mutational spectrum of β-thal in Balearic Islands, thus enabling to establish the best mutation detection strategy to use for genetic diagnosis, genetic counselling programmes and prenatal diagnosis.

Materials and methods

Population samples

Consecutive blood samples from 22,713 anonymous, unrelated individuals, over 6 years old were collected in the primary care laboratory of three hospitals – 14,175 in Son Dureta (Majorca), 5893 in Can Misses (Ibiza) and 2645 in Mateu Orfila (Minorca) – which collect samples from all over the three Balearic Islands (∼1 million inhabitants altogether).

A simplified flow chart based on MCV, MHC, iron metabolism, HbA2 and HbF values was used for carrier screening (7).

Methods

Haematological indices were obtained with an automated cell counter (ADVIA 120 Hematology System, Siemens Healthcare Diagnostics, Deerfield Road, IL). To discard iron deficiency in samples with microcytosis, a Hitachi Modular 917 analyser (Roche Diagnostic, Mannheim, Germany) was used.

HbA2 and HbF levels were performed by high-performance liquid chromatography (HPLC) (HA-8160, Menarini Diagnostic, Florence, Italy). Detected haemoglobin (Hb) variants were identified using the Bio-Rad Variant II HPLC system (Bio-Rad Laboratories, Munich, Germany) and confirmed by sequencing.

DNA was extracted from peripheral blood using the QIAamp Blood kit (Qiagen, Crawley, UK). Five β-globin gene mutations: βCDS9, βCD39, βCD37, βIVS-I-110, βIVS-I-1 and βIVS-I-6 were screened by the LightCycler PCR method (Roche Diagnostic) (8).

In samples where none of these mutations was identified, β-globin gene was sequenced with primers described by Clark and Thein (9) using Big Dye® Terminator Cycle Sequencing kit v.3.1 and ABI PRISM® 3100 Genetic Analyser (Applied Biosystems, Foster City, CA).

Samples with increased HbF levels in adulthood (>3.5%) and normal (or reduced) HbA2 levels (<3.5%) were screened for the Spanish (δβ)0-thalassemia using a GAP-PCR method (10).

Statistical analysis

Statistical comparison of mutation frequencies between populations was performed using SPSS v.12.0 (SPSS Software Inc., Chicago, IL).

Patterns of geographical variation were examined using Pearson correlation coefficient between geographical parameters (longitude) and relative frequency of β-thal mutations in 17 Mediterranean countries plus Balearic data.

Results

Prevalence of β-thal in Balearic Islands

A total of 22,713 samples were screened, 175 of which turned out to be β-thal carriers (see haematological characteristics of carriers and controls in Table 1). Therefore, overall prevalence of β-thal trait in Balearic Islands was 0.77%. However, significant differences were found between islands. Majorca and Ibiza showed similar values (0.59% and 0.46%, respectively), whereas Minorca exhibited a much higher prevalence (2.46%) (Fig. 1).

Different structural Hb variants were also found: eight individuals with sickle cell trait (HbS) and three heterozygotes for HbC, HbE and HbLepore, respectively.

Identification of β-globin gene mutations

Screening for common mutation by LightCycler PCR method enabled detection of 80.5% mutations (141 subjects), and 13 β-thalassemic chromosomes were identified by direct sequencing (7.5%). Seven individuals (4.0%) were tested for, and found to have, Spanish type δβ0-thalassemia. For the remaining 8.0% (14 subjects), the mutation could not be identified (Table 2).

Eleven mutations were detected in all; the most common was CD39 (C>T), with a frequency of 61.1%; followed by IVS-I-110 (G>A) (12.0%), and IVS-I-6 (T>C) and IVS-I-1 (G>A) (3.4% both). Other mutations (<3%) were: −32 (C>T), CD6 (−A), CD8/9 (+G), CD37 (G>A), CD44 (−C), IVS-II-705 (T>G) and IVS-II-745 (C>G) (Table 2).

A comparison of the β-thal mutation spectrum between different islands (Table 2 and Fig. 1) showed significant differences (p < 0.001). In Minorca, one mutation CD39 (C>T) was found in 93.8% of β-thal chromosomes, whereas more heterogeneity was
Table 1. Haematological parameters in β-thalassemia carriers and control population (X ± SD)

<table>
<thead>
<tr>
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<th>Control population (n = 674)</th>
<th>β-thal carriers (n = 175)</th>
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<tbody>
<tr>
<td>Red blood cells⁶ (×10⁶/ul)</td>
<td>4.7 ± 0.5 (4.6–4.8)</td>
<td>5.6 ± 0.6 (3.1–7.3)</td>
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<tr>
<td>Haemoglobin⁶ (g/dl)</td>
<td>14.2 ± 1.5 (14.1–14.3)</td>
<td>11.8 ± 1.3 (6.9–15.2)</td>
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<tr>
<td>MCV⁶ (fl)</td>
<td>87.4 ± 4.0 (87.1–87.7)</td>
<td>64.6 ± 3.9 (55.4–77.7)</td>
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<tr>
<td>MCH⁶ (pg)</td>
<td>30.1 ± 1.3 (29.9–30.2)</td>
<td>20.6 ± 1.7 (14.3–26.6)</td>
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<tr>
<td>HbF⁶ (%)</td>
<td>0.3 ± 0.1 (0.2–0.3)</td>
<td>1.1 ± 1.0 (0.2–7.7)</td>
</tr>
<tr>
<td>HbA₂ (%)</td>
<td>3.0 ± 0.2 (2.9–3.1)</td>
<td>5.0 ± 0.8 (2.1–6.3)</td>
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⁶Values based on a control population of 674 individuals.

found in the other islands, especially Majorca with 11 different mutations, although the most prevalent was also CD39 (C>T) (45.8% prevalence), followed by IVS-I-110 (G>A) (12%). In Ibiza, five mutations were detected, with IVS-I-110 (G>A) (37%) as the most common, unlike the other islands.

Comparison with other Mediterranean countries

Correlation between frequencies of the main mutations [CD39 (C>T) and IVS-I-110 (G>A)] in β-thal chromosomes and geographical parameters (longitude) showed a decreasing west–east frequency for CD39 (C>T) (r = −0.781) (Fig. 2a), but an increasing west–east distribution tendency (r = 0.682) for IVS-I-110 (G>A) (Fig. 2b). Regression values were significant in both cases (p = 0.00013 and p = 0.00182, respectively).

Discussion

No population studies for β-thal have been carried out in the different Balearic Islands which have special interest because of their insularity: founder effects and genetic drift are likely and genetic studies show historical–demographic differences between the three islands are reflected in their gene pool; emphasizing the need to document frequencies of mutant alleles in each island, to facilitate carrier detection programmes and downstream healthcare initiatives.

In Spain, β-thal is relatively frequent (0.3–0.4% estimated overall carriers prevalence), although with significant differences between regions (reviewed in Ref. 4). The Balearic population has twice the average prevalence of heterozygous β-thal (~0.8%), which is higher than most Spanish regions but still clearly lower than neighbouring Mediterranean countries [ranging from 1.4% (Algeria) to 5.5% (Greece) (11–13)].

One might wonder why β-thal frequencies are much lower in Spain than in other Mediterranean countries. Hence, we must consider the widely postulated hypothesis that selection by malaria is the evolutionary force responsible for current prevalence and distribution of β-thalassemia, because of selective heterozygote advantage against malaria for carriers of erythrocyte defects such as glucose-6-phosphate-dehydrogenase (G6PD) deficiency or different haemoglobinopathies (reviewed in Ref. 14). Thus the low prevalence of β-thal in the Iberian Peninsula could be because of a
smaller effect of malaria in these populations; yet it is
difficult to assess because of the impossibility of quan-
tifying the effect of this disease in the population in
ancient times. Besides, other evolutionary forces could
also have contributed to this difference.

These results confirm Minorca as the Spanish popu-
lation with the highest \( \beta \)-thal prevalence (2.5\%) (15).
Serious successive malaria epidemics are known to
have occurred there in the past (e.g. in the eighteenth
century) (16); thus the fact that both \( \beta \)-thal and G6PD
deficiency, together with the O allele (ABO) – which
also seems to confer resistance to malaria para-
sites (14) – reach, in Minorca, the highest frequencies
in Spain (17, 18), would support the hypothesis that
the \( \beta \)-thal trait frequency reflects a greater impact of
malaria in the Minorca population.

Regarding molecular analysis, two mutations, CD39
(C>G) and IVS-I-110 (G>A) – probably the oldest
ones – are the most observed in Balearic Islands,
as in other Mediterranean populations. CD39 (C>G)
is predominant in the Western Mediterranean (North
African and Southern European countries) with a fre-
quency of approximately 30–40\%, whereas in the East-
ern Mediterranean area the most common is IVS-I-
110 (G>A). Balearic population fitted the expected
distribution in the opposite clines described for both
mutations in the Mediterranean.

Noteworthy is the high island-specific molecular
heterogeneity of \( \beta \)-thal mutations in the three Balearic
Islands. Majorca is the most heterogeneous, with 11
\( \beta \)-thal mutations. The four most common mutations
show similar frequencies to Spanish Mediterranean
populations, except IVS-I-6 (T>C) (4.8\%) which has
a lower frequency. Of the rare mutations, CD6 (−A)
(2.4\%) is significantly different from the unusually high
frequency (20.4\%) described previously in Majorcan
patients (4). Three mutations [CD44 (−C), −32 (C>T)
and IVS-II-705 (T>G)], not previously reported in
Spain are observed. CD44 (−C) is found in Middle
Eastern and North African populations. Its presence
in Majorca could be because of migratory phenomena,
either recent (e.g. current immigration from Maghreb)
or historical (e.g. Jewish Diaspora or Phoenician trade
routes). The other mutations are rare everywhere.

In Minorca, the molecular pattern is very different.
IVS-I-110 (G>A) and CD8/9 (+G) are present only
in one individual each, whereas CD39 (C>T) reaches
the amazing frequency of 93.8\%. Only in one study
on Sardinia were similar values described (19). This
mutation appears to be of Roman origin, diffused
the spectrum of influence (21, 23–27), the Phoenician–Carthaginian in the Mediterranean has been linked to Phoenician Phoenicians might have been) and that its distribution population is considered most representative of what βαimportant differences in genetic markers, for example, mtDNA, also evidence (Gαsize (22). However, considering the high IVS-I-110 (G>A) frequency in each of these three islands.

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**References**


throughout the former Roman Empire. No historical reasons explain why these two populations (Minorca and Sardinia) present this unusual mutational homogeneity in β-thal chromosomes, therefore owing to their insular nature this fact is probably related to founder effect and/or genetic drift.

In Ibiza, the four most common mutations plus the rare −32 (C>T) are detected. This is the only Western Mediterranean population where the most frequent β-thal mutation is IVS-I-110 (G>A), with a frequency (37%) similar to Eastern Mediterranean populations, such as Lebanon (33–62%) (20, 21), Turkey (42%) and Greece (44%) (7). This mutation is believed to have arisen in Turkey and spread into surrounding countries owing to colonization by Turks, Greeks and Phoenicians. The differential pattern of β-thal mutation frequencies in Ibiza could be because of genetic drift, because historically Ibiza was an isolated, consanguineous population with a reduced effective population size (22). However, considering the high IVS-I-110 (G>A) mutation frequency in Lebanon (their current population is considered most representative of what Phoenicians might have been) and that its distribution in the Mediterranean has been linked to Phoenician influence (21, 23, 24–27), the Phoenician–Carthaginian origin of the Ibiza population seems the main cause of the spectrum of β-thal mutations on this island. Other genetic markers, for example, mtDNA, also evidence this origin (5).

In conclusion, the three Balearic Islands show important differences in β-thal patterns which can be explained by a combination of historical–demographic characteristics and action of evolutionary factors such as founder effect, genetic drift and, probably, selection by malaria. Knowledge of the mutational spectrum in the Balearic Islands will enable to optimize mutation detection strategy for genetic diagnosis of β-thal carriers in each of these three islands.