The molecular spectrum and distribution of haemoglobinopathies in Cyprus: a 20-year retrospective study

Petros Kountouris1, Ioanna Kousiappa1, Thessalia Papasavva1, George Christopoulos1, Eleni Pavlou1, Miranda Petrou1, Xenia Feleki1, Eleni Karitzie1, Marios Phylactides1, Pavlos Fanis1, Carsten W. Lederer1, Andreani R. Kyrri2, Eleni Pavlou1, Miranda Petrou1, Christa Makariou1, Christina Ioannou2, Loukas Kythreotis2, Georgia Hadjilambi2, Nicoletta Andreou2, Evangelia Pangalou1, Irene Savvidou3, Michael Angastiniotis4, Michael Hadjigavriel5, Maria Sitarou6, Anna Kolnagou7, Marina Kleanthous1 & Soteroula Christou3

Haemoglobinopathies are the most common monogenic diseases, posing a major public health challenge worldwide. Cyprus has one of the highest prevalences of thalassaemia in the world and has been the first country to introduce a successful population-wide prevention programme, based on premarital screening. In this study, we report the most significant and comprehensive update on the status of haemoglobinopathies in Cyprus for at least two decades. First, we identified and analysed all known 592 β-thalassaemia patients and 595 Hb H disease patients in Cyprus. Moreover, we report the molecular spectrum of α-, β- and δ-globin gene mutations in the population and their geographic distribution, using a set of 13824 carriers genotyped from 1995 to 2015, and estimate relative allele frequencies in carriers of β- and δ-globin gene mutations. Notably, several mutations are reported for the first time in the Cypriot population, whereas important differences are observed in the distribution of mutations across different districts of the island.

The haemoglobin (Hb) molecule is responsible for binding and transport of oxygen and carbon dioxide by red blood cells and is critical for their shape, integrity and half-life. The Hb protein complex consists of two α-like chains, encoded by genes in the α-locus on chromosome 16 (RefSeq ID: NG_000006), namely ζ (HBZ), α1 (HBA1) and α2 (HBA2), and two β-like chains, encoded by genes in the β-locus on chromosome 11 (RefSeq ID: NG_000007), namely ε (HBE), Aγ (HBG1), Gγ (HBG2), δ (HBD) and β (HBB). Haemoglobinopathies are caused by mutations in the two globin gene clusters and are characterised by a reduced or absent synthesis of globin chains in the case of the thalassaemia syndromes, mainly α- and β-thalassaemia, or by defects in the Hb protein structure in the case of structural Hb variants, such as the Hb S that causes sickle-cell disease. To date, more than 1865 disease-causing mutations have been reported, affecting different levels of gene regulation and expression1.

Haemoglobinopathies are the most common monogenic diseases in the world1 and are prevalent in former malaria regions in the Mediterranean, the Middle-East, South-East Africa and Sub-Saharan Africa3. Cyprus is an Eastern Mediterranean island where an exceptionally high incidence of thalassaemia poses a major public health challenge. More specifically, the prevalence of β-thalassaemia patients was estimated to be around 1 in 10004, while the β-thalassaemia carrier rate was recently estimated to be around 12%5, with earlier estimates in

1Molecular Genetics Thalassaemia, The Cyprus Institute of Neurology and Genetics, 6 International Airport Ave., 2370 Nicosia, Cyprus. 2Thalassemia Screening Laboratory, Thalassemia Center, Archbishop Makarios III Hospital, 1474 Nicosia, Cyprus. 3Thalassemia Center, Archbishop Makarios III Hospital, 1474 Nicosia, Cyprus. 4Thalassaemia International Federation, 31 Ifígenias Street, 2007 Strouvas, Nicosia, Cyprus. 5Thalassemia Center, Limassol General Hospital, 3304 Limassol, Cyprus. 6Thalassemia Center, Larnaca General Hospital, 6301 Larnaca, Cyprus. 7Thalassemia Center, Paphos General Hospital, 8100 Paphos, Cyprus. Correspondence and requests for materials should be addressed to P.K. (email: petrosk@cing.ac.cy)
the range of 15–18%6, one of the highest in the world. In addition, the α-thalassaemia carrier rate was estimated to be around 20%7, although earlier studies using electrophoresis estimated it to be about 10–12% of the population8,9. Cyprus was the first country to introduce a successful population-wide prevention programme for β-thalassaemia, based on premarital screening, and, as a result, the annual birth rate has decreased to less than five cases from an expected 30–50.10

α-thalassaemia is characterised by a decrease or complete absence of expression from one or more of the four α-globin genes and may be brought about by a deletion or a nondeletion mutation in the α-globin genes. Mutations are divided into two major classes: (a) α0-thalassaemia mutations, which delete both α-globin genes on the same chromosome, and (b) α-α-thalassaemia mutations, which delete or deactivate only one of the α-globin genes. As a result, four clinical conditions of increased severity are recognised, based on the number of α-globin genes affected11,12: (a) α-thalassaemia silent carrier, in which only one α-globin gene is affected by a deletion (–/αα) or a nondeletion (αND/αα) and which is mainly asymptomatic, (b) α-thalassaemia trait, in which only two α-globin genes are functional, either in cis (–/αα) or in trans (–/α0 or α0/αα), and which usually results in mild anaemia, (c) Hb H disease, in which there is only one functional α-globin gene (–/αα or –/αNDα), and (d) Hb Bart’s hydrops fetalis, in which all α-globin genes are deleted (–/–), leading to severe anaemia and, usually, death in utero or shortly after birth. The spectrum of α-thalassaemia mutations has been well-documented over the last decades13, with more than 230 mutations currently reported in the public lthaGenes database1. In Cyprus, the α-thalassaemia carrier rate and the relative allele frequencies were previously determined by screening 495 random cord blood samples2. The –α.3.7 deletion is the most common α-globin mutation, accounting for 72.8% of all α-globin mutations, while the most common α mutations, specifically α20.5 and –MED I, account for a combined 7.8% of all α-globin mutations and about 0.8% of the population.

Consequently and despite the high prevalence of α-thalassaemia carriers in Cyprus, the risk for Hb Bart’s hydrops fetalis is relatively low, due to the low prevalence of α mutations in the population. In contrast, the number of Hb H disease patients is higher, but no study has ever determined the Hb H disease prevalence in the population, an omission that is all the more striking given that a wide range of severe phenotypic characteristics have been observed, particularly in the nondeletional type of the disease14,15.

β-thalassaemia is characterised by the reduced synthesis (β+) or absence (β0) of the β-globin chains in the Hb molecule, resulting in accumulation of unbound α-globin chains that precipitate in erythrocytic precursors in the bone marrow and in the mature erythrocytes, leading to ineffective erythropoiesis and peripheral haemolysis16. It is mainly caused by single nucleotide substitutions, small deletions or insertions within the β-globin gene or its immediate flanking sequence and, rarely, by large deletions17,18. To date, more than 350 β-thalassaemia mutations have been reported in the lthaGenes database1. In Cyprus, the most common β-thalassaemia mutation is IVS I-110 (G>A), with a frequency of 74–80%, followed by three other alleles, specifically IVS II-745 (C>G), IVS I-6 (T>C), IVS I-1 (G>A), with frequencies of 5–8%19.

In adult life, Hb A (α2β2) is the major Hb component with Hb A2 (α2γ2) represented in a fraction of 2.5–3.5% and with traces (<1%) of Hb F (α2γ2). The percentage of Hb A2 is usually higher in β-thalassaemia carriers, because of the reduced production of Hb A, while it is usually lower for Hb H disease patients. Therefore and although β-thalassaemia has no clinical significance, mutations in the β-globin gene interfere with typical thalassaemia phenotypic characteristics, affecting population screening for thalassaemia. Thus, investigation of β-globin gene mutations in the Cypriot population is important to avoid misdiagnosis for thalassaemia carriers20. In a previous study21, the carrier frequency for mutated β-globin chromosomes has been demonstrated as close to 1.26% and the spectrum of observed β-globin gene mutations was reported for the Cypriot population.

In addition to the thalassaemias, several less prevalent structural Hb variants have been identified in the Cypriot population22. Nine structural variants concerning the β-globin chains and three concerning the α-globin chains have been identified, with the most common being Hb S (0.2%), Hb D-Punjab (0.02%), Hb Lepore Boston-Washington (0.03%) for the β-globin chain and Hb Setif (0.1%) for the α-globin chain. Recently, a novel δ-globin chain variant (Hb A2-Famagusta) was discovered in four distinct families in Cyprus23, while other δ-globin variants have been observed in the past24, with Hb A2-Yialousa being the most prevalent.

This article reports (a) the molecular spectrum and geographic distribution of all known β-thalassaemia patients and Hb H disease patients in Cyprus, (b) the molecular spectrum of α, β- and δ-globin gene mutations in their population and their geographic distribution, and (c) an updated, more precise estimation of the relative allele frequencies in carriers of β- and δ-globin gene mutations. In this retrospective investigation, we retrieved and analysed genotypic characteristics of samples isolated during the last 20 years from 592 β-thalassaemia patients, 595 Hb H disease patients and 13824 carriers of α-, β- and δ-globin gene mutations. Therefore, this study represents the most significant and comprehensive update on the status of haemoglobinopathies in Cyprus for at least two decades, providing comprehensive evidence for the success and critical information for the improvement of the population screening programme.

Materials and Methods

Ethics Statement. The study is in accordance with the guidelines and regulations of the Cyprus legislation and National Bioethics Committee. All genetic and personal information used throughout this study were collected as part of the routine diagnostic services at the Cyprus Institute of Neurology and Genetics (CING) from 1995 to 2015, after the request of the participants and in accordance with the CING regulations, whereas no additional data were collected or stored for this research investigation. All subjects were de-identified in compliance with the FDA Guidance Document “Informed Consent for In Vitro Diagnostic Device Studies Using Leftover Human Specimens that are Not Individually Identifiable” issued in April 2006 and is exempt from IRB review, as also confirmed by the Cyprus National Bioethics Committee. In addition, the study is in accordance with the guidelines provided in 2002 by the Council for International Organizations of Medical Sciences (CIOMS) in collaboration with the World Health Organization (WHO), as well as with the Working Party document 131
Table 1. Description of datasets. aIncluding related individuals. bAfter filtering related individuals with the same genotype. cNumber of individuals per district, after filtering those in unidentified locations. dAge on 10 Dec 2015.

DNA isolation and genotyping of αα, ββ-, and δδ-globin genes. Genomic DNA was extracted from peripheral blood using the Gentra Puregene Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol and kept at −80 °C for long-term storage. For genotyping of the causative pathological ββ-globin mutations, we used the single-tube amplification refractory mutation system-Polymerase Chain Reaction (ARMs-PCR) methodology. Seven mutations common to the Cypriot population were tested, specifically IVS I-110 (G>A), IVS I-6 (T>G), IVS I-1 (G>A), IVS II-745 (C>G), CD 39 (C>T), -87 (C>G) and CD 5 (-CT). When no mutation was detected by the ARMS-PCR, the ββ-globin gene was examined by performing PCR and Sanger sequencing using an ABI 3130xl Genetic Analyzer (Applied Biosystems-Life Technologies, USA). Deletion-type mutations were tested using the multiplex ligation-dependent probe amplification method (MLPA) by utilising the SALSA MLPA probemix P102 HBB protocol (MRM, Holland).

The samples of ββ-thalassaemia patients, Hb H disease patients and carriers of αα-globin gene mutations were investigated for αα-globin deletions and/or point mutations by gap-PCR genotyping assays. The αα-thalassaemia screening panel consisted of deletions and point mutations representing the common αα-thalassaemia determinants encountered previously in Cyprus, specifically αα3.7, triplicated αα (αααααα or anti-αα3.7), -MED I, −αα20.5, IVS I-1 (-5bp), Poly(A) AATAAA >AAATGAA and Hb Agrinio. In the case of Hb H disease patients and when no mutation was detected by the gap-PCRs, the αα-globin genes were studied by sequencing, as detailed
Results and Discussion
Carriers of α-globin gene mutations. From 1995 to 2015, 9287 carriers of α-globin gene mutations were genotyped at the CING. After removing related individuals with identical genotype, the remaining 8412 carriers were used to analyse the molecular spectrum and distribution of α-globin gene mutations, shown in Table 2. However, we do not report the relative allele frequencies, because the dataset of α-globin gene mutation carriers referred to the CING for genotyping is not a random representation of the carrier population, particularly owing to the underrepresentation of silent α-thalassaemia carriers in the dataset, such as individuals with genotypes αα/α3.7 or αα/α4.2 and CD 108 (-C), and two α-locus and three cases with unknown deletions involving both genes, namely Hb Icaria and Hb Stanleyville-II, are reported for the first time in Cyprus. More specifically, three α-thalassaemia mutations, namely --SEA, --α4.2 and CD 108 (-C), and two α-chain structural variants, namely Hb Icaria and Hb Stanleyville-II, are reported for the first time through this study, whilst three cases with α3 deletions in the erythroid-specific DNAse I hypersensitive site MCS-R2 (HS40) were detected and are currently under investigation to determine the precise breakpoints. Moreover, four cases with unknown duplications of the α-locus and three cases with unknown deletions involving both genes, α2 and α1, were detected and are currently under investigation.

In addition, the geographic distribution of α-globin gene mutations reveals statistically significant differences between districts. Notably, the --MED I deletion is more prevalent in Larnaca/Famagusta, where the --α3.7 allele is less prevalent. Moreover, the severe IVS I-1 (-5 bp) allele is more prevalent in Larnaca/Famagusta and Paphos, while a higher prevalence of the --α20.5 deletion is observed in Nicosia.

Table 2. Allele frequencies in 8412 carriers of α-globin gene mutations in Cyprus and in individual districts. n: number of alleles; NA: Not available.

| Common name | HGVS name | InhAI3 | Cyprus-wide | Per district (%) (95% CI) |
|--------------|-----------|--------|-------------|--------------------------|
| n | Larnaca/ Famagusta (n = 2415) | Limassol (n = 2184) | Nicosia (n = 4145) | Paphos (n = 683) |
| α3.7 (type I) | NG_000006.1.g:34164_37967del3804 | 300 | 6363 | 50.60 (48.58, 52.61) | 69.28 (67.29, 71.20) | 64.32 (62.84, 65.77) | 67.35 (63.67, 70.83) |
| --MED I | NG_000006.1.g:24664_41064del e116401 | 312 | 1468 | 23.44 (21.77, 25.19) | 9.98 (8.77, 11.33) | 12.3 (11.33, 13.35) | 8.78 (6.82, 11.22) |
| IVS1-1 (-5 bp) | HBA2:c.95 +5 to +2_95 + 6delTGAGG | 359 | 1458 | 17.02 (15.55, 18.59) | 12.59 (11.24, 14.07) | 13.29 (12.28, 14.37) | 17.57 (14.83, 20.68) |
| --α20.5 | NG_000006.1.g:15164_37864del e122701 | 314 | 382 | 2.98 (2.36, 3.76) | 2.61 (2.00, 3.39) | 4.95 (4.32, 5.66) | 1.76 (0.95, 3.14) |
| Poly(A) AATAAA > AATGAA | HBA2;c.92A>G | 425 | 212 | 2.15 (1.63, 2.84) | 2.79 (2.16, 3.60) | 1.93 (1.54, 2.41) | 0.44 (0.11, 1.39) |
| Triplicated α (anti-3.7) | NA | 2561 | 102 | 0.99 (0.65, 1.50) | 0.6 (0.33, 1.04) | 1.09 (0.80, 1.46) | 1.02 (0.45, 2.20) |
| Hb Agrinio | HBA2:c.89T>C | 356 | 86 | 1.20 (0.82, 1.74) | 0.92 (0.57, 1.44) | 0.58 (0.38, 0.87) | 1.46 (0.75, 2.77) |
| Poly(A) AATAAA > AATGAG | HBA2;c.94A>G | 424 | 50 | 0.75 (0.46, 1.20) | 0.32 (0.14, 0.69) | 0.46 (0.28, 0.73) | 0.73 (0.27, 1.80) |
| Hb Setif | HBA2;c:283G>T | 673 | 39 | 0.17 (0.05, 0.45) | 0.37 (0.17, 0.75) | 0.51 (0.32, 0.79) | 0.73 (0.27, 1.80) |
| CD 108 (-C) | HBA1:c.327delC | 398 | 14 | 0.25 (0.10, 0.57) | 0.09 (0.02, 0.37) | 0.10 (0.03, 0.27) | 0.15 (0.01, 0.95) |
| Hb Icaria | HBA2;c:242T>A | 419 | 11 | 0.04 (0.00, 0.27) | 0.05 (0.00, 0.30) | 0.22 (0.11, 0.43) | – |
| --MED II | NG_000006.1.g:10864_40864del e130001 | 313 | 8 | 0.25 (0.10, 0.57) | 0.05 (0.00, 0.30) | 0.02 (0.00, 0.16) | – |
| --α4.2 | NA | 5 | 0.08 (0.01, 0.33) | 0.09 (0.02, 0.37) | 0.02 (0.00, 0.16) | – |
| Hb Fontainebleau | HBA2;c:64G>C | 481 | 4 | – | 0.14 (0.04, 0.44) | 0.02 (0.00, 0.16) | – |
| Unknown duplications | NA | NA | 4 | – | 0.09 (0.02, 0.37) | 0.05 (0.01, 0.19) | – |
| HS40 deletions | NA | NA | 3 | 0.04 (0.00, 0.27) | – | 0.05 (0.00, 0.16) | – |
| Unknown deletions involving α2 and α4 | NA | NA | 3 | 0.04 (0.00, 0.27) | 0.05 (0.00, 0.30) | 0.02 (0.00, 0.16) | – |
| --SEA | NG_000006.1.g:26264_45564del e119301 | 309 | 2 | – | – | 0.05 (0.00, 0.16) | – |
| Hb Stanleyville-II | HBA2;c:237C>A;237C>G | 626 | 1 | – | – | 0.02 (0.00, 0.16) | – |
| Common name | HGVS name | IbaII^1 | Cyprus-wide | Per district (95% CI) |
|-------------|-----------|--------|-------------|----------------------|
|             |           |        | n | Frequency (%) | Larnaca/Famagusta | Limassol (n = 525) | Nicousia (n = 722) | Paphos (n = 153) |
| IVS I-110 (G>A) | HBB:c.93-21G>A | 113 | 1845 | 79.01 (77.29, 80.64) | 74.49 (75.48, 83.00) | 74.48 (70.48, 78.11) | 77.42 (74.16, 80.39) | 92.16 (86.39, 95.70) |
| IVS I-6 (T>C) | HBB:c.92 +6T>C | 111 | 144 | 6.34 (5.40, 7.42) | 5.98 (4.08, 8.63) | 6.48 (4.59, 9.02) | 7.34 (5.60, 9.55) | 3.27 (1.21, 7.86) |
| IVS I-1 (G>A) | HBB:c.92 +1G>A | 101 | 140 | 6.00 (5.08, 7.06) | 6.84 (4.80, 9.62) | 8.00 (5.89, 10.74) | 5.40 (3.92, 7.38) | 1.96 (0.51, 6.07) |
| IVS II-745 (C>G) | HBB:c.316-106C>G | 214 | 96 | 4.11 (3.36, 5.02) | 4.70 (3.04, 7.14) | 5.33 (3.64, 7.71) | 4.02 (2.75, 5.79) | 1.96 (0.01, 0.89) |
| CD 39 (CAG>TA) | HBB:c.118C>T | 142 | 42 | 1.80 (1.32, 2.45) | 0.21 (0.01, 1.38) | 1.90 (0.97, 3.59) | 3.05 (1.95, 4.65) | – |
| Hb S | HBB:c.20A>T | 824 | 22 | 0.94 (0.61, 1.45) | 0.85 (0.27, 2.32) | 1.14 (0.47, 2.60) | 0.97 (0.43, 2.08) | 0.65 (0.03, 4.14) |
| -87 C>G | HBB:c.137C>G | 10 | 12 | 0.51 (0.28, 0.92) | 0.43 (0.07, 1.71) | 0.76 (0.24, 2.08) | 0.42 (0.11, 1.32) | – |
| CD 44 (-C) | HBB:c.135delC | 151 | 10 | 0.43 (0.22, 0.81) | – | 1.14 (0.47, 2.60) | 0.55 (0.18, 1.51) | – |
| Hb Lepore Boston-Washington | NG_000007.3:g.636+32_71046del7414 | 1399 | 4 | 0.17 (0.05, 0.47) | 0.21 (0.01, 1.38) | 0.19 (0.01, 1.23) | 0.14 (0.01, 0.89) | – |
| IVS II-848 (C>A) | HBB:c.316-3C>A | 224 | 4 | 0.17 (0.05, 0.47) | 0.85 (0.01, 1.38) | – | – | – |
| IVS II-1 (G>A) | HBB:c.315 +1G>A | 200 | 3 | 0.13 (0.03, 0.41) | 0.21 (0.01, 1.38) | – | 0.14 (0.01, 0.89) | – |
| Sicilian (δ) | NG_000007.3:g.6443_36_77738del113403 | 1507 | 3 | 0.13 (0.03, 0.41) | – | 0.38 (0.07, 1.52) | – | – |
| CAP +33 (C>G) | HBB:c.18C>G | 39 | 2 | 0.09 (0.01, 0.34) | – | 0.19 (0.01, 1.23) | 0.14 (0.01, 0.89) | – |
| -101 (C>T) | HBB:c.151C>T | 1030 | 3 | 0.04 (0.002, 0.28) | – | 0.14 (0.01, 0.89) | – | – |
| Hb City of Hope | HBB:c.208G>A | 817 | 1 | 0.04 (0.002, 0.28) | 0.21 (0.01, 1.38) | – | – | – |
| Hb D-Punjab | HBB:c.364G>C | 1217 | 1 | 0.04 (0.002, 0.28) | – | 0.14 (0.01, 0.89) | – | – |
| Hb Knossos | HBB:c.82G>T | 91 | 1 | 0.04 (0.002, 0.28) | – | 0.14 (0.01, 0.89) | – | – |

Table 3. Allele frequencies in 2335 carriers of β-globin gene mutations in Cyprus and in individual districts. n: number of alleles.

Carriers of β-globin gene mutations. Population screening in Cyprus was established mainly to prevent β-thalassaemia, which is usually a more severe disorder than the Hb H disease. For this reason, prenatal diagnosis has been offered for couples at risk for an affected β-thalassaemia birth. The list of all couples participating in prenatal testing is a random representation, with regards to genotype, of the β-thalassaemia carrier population in Cyprus, because it comprises unrelated individuals without any bias for mild or severe mutations. Thus, from the 4700 carriers of β-globin gene mutations genotyped at the CING during 1995–2015, we have selected all couples participating in prenatal testing for the calculation of the carrier frequencies, resulting in 2335 individuals, and the results are shown in Table 3. The most common β-thalassaemia mutation in the population is IVS I-110 (G>A), with a frequency of 79.01%, followed by mutations IVS I-6 (T>C), IVS I-1 (G>A) and IVS II-745 (C>G) with frequencies of 6.34%, 6.00% and 4.11%, respectively. These frequencies are in agreement with values reported in an earlier study, but the present study provides both a more precise estimate through its much larger sample size and a critical update to numbers that date back 23 years.

Most geographical differences observed in β-globin gene mutation frequencies are small and not statistically significant, as demonstrated by the 95% CI shown in Table 3. Specifically, the frequency of the IVS I-110 (G>A) mutation is significantly higher in Paphos, albeit with a smaller sample size. In addition, small differences are observed in Limassol, with slightly higher frequencies for alleles IVS I-1 (G>A) and IVS II-745 (C>G), and Nicousia, with a slightly higher frequency for the IVS I-6 (T>C) allele.

Notably, several mutations are reported here for the first time in the Cypriot population. More specifically, five β-thalassaemia mutations, namely -87 (C>G), CD 44 (-C), IVS II-1 (G>A), -101 (C>T) and Sicilian (δ) Sicilian, and the β-chain structural variant Hb City of Hope have not been observed in earlier studies, whereas the mutation IVS II-848 (C>A) has been reported only in the Turkish Cypriot population in the past. Apart from the mutations listed in Table 3, other mutations were observed in individuals genotyped at the CING, but not included in the final dataset that was based on individuals participating in prenatal diagnosis. These mutations are rare in the population, with frequencies of 0.5% or lower, and include CD 8 (-AA), Hb Beirut, Hb Serres, Hb Limassol, Hb Nicousia, Hb O-Arab, Hb G-Accra that have been observed in the population in the past, but also mutations that are reported in the Cypriot population for the first time, namely Hb C, Hb E, -30 (T>A), CD 36/37(−T) and -92 (C>T).

Carriers of δ-globin gene mutations. 504 individuals carrying at least one δ-globin gene mutation were genotyped at the CING from 1995 to 2015. After removing related individuals, a dataset of 428 carriers was available to calculate the carrier frequencies shown in Table 4. The most common δ-globin gene mutation in Cyprus was CD 27 (GCC>TCC), resulting in Hb A2-Yialousa, with a frequency of 48.12%, followed by CD 4 (ACT>ATT), Hb A2-Yokoshiba, Hb A2-Pelendri and IVS II-897 (A>G) with frequencies of 19.87%, 11.26%, 6.84% and 5.96%, respectively. Notably, three δ-globin chain variants were observed in the Cypriot population for the first time, namely Hb A2-NYU, Hb A2 and Hb A2-Etolia.

Differences in the geographic distribution of β-globin gene mutations are observed across different districts. Hb A2-Yialousa is particularly prevalent in the Larnaca/Famagusta district, while the CD 4 (ACT>ATT) allele is
Table 4. Allele frequencies in 428 carriers of δ-globin gene mutations in Cyprus and in individual districts. 

| Common name | HGVS name | IthID① | Cyprus-wide | Per district (%) (95% CI) | Larnaca/Famagusta (n = 138) | Limassol (n = 94) | Nicosia (n = 160) | Paphos (n = 19) |
|-------------|------------|--------|-------------|--------------------------|-----------------------------|-----------------|-----------------|-----------------|
| Hb Aβ2-Yaloussa | HBD.c.82G>T | 1332 218 | 48.12 (43.45, 52.83) | 59.42 (50.72, 67.59) | 24.47 (16.44, 34.62) | 49.38 (41.43, 57.35) | 36.84 (17.23, 61.37) |
| CD 4 (ACT>ATT) | HBD.c.14C>T | 1331 80 | 19.87 (16.35, 23.91) | 9.42 (5.32, 15.88) | 28.72 (20.09, 39.12) | 22.50 (16.44, 29.91) | 36.84 (17.23, 61.37) |
| Hb Aβ2-Yokoshima | HBD.c.77G>A | 1360 51 | 11.26 (8.57, 14.62) | 13.77 (8.70, 20.92) | 20.21 (19.30, 30.01) | 6.88 (3.65, 12.27) | 5.26 (0.28, 28.11) |
| Hb Aβ2-Pelendri | HBD.c.425T>C | 1392 31 | 6.84 (4.77, 9.68) | 3.62 (1.34, 8.68) | 15.96 (9.50, 25.27) | 5.00 (2.34, 9.95) | – |
| IVS II-897 (A>G) | HBD.c.316-2A>G | 2473 27 | 5.96 (4.04, 8.66) | 7.25 (3.72, 13.27) | 7.45 (3.30, 15.25) | 3.75 (1.53, 8.35) | 5.26 (0.28, 28.11) |
| Hb Aβ2-Famagusta | HBD.c.60C>A | 2292 8 | 1.77 (0.82, 3.59) | 1.45 (0.25, 5.67) | – | 1.88 (0.49, 5.81) | 15.79 (4.17, 40.49) |
| Hb Aβ2-Troodos | HBD.c.349C>T | 1384 5 | 1.10 (0.41, 2.71) | 1.45 (0.25, 5.67) | 1.06 (0.06, 6.62) | 1.25 (0.22, 4.91) | – |
| IVS 1-2 (T>C) | HBD.c.92+2T>C | 1334 5 | 1.10 (0.41, 2.71) | 0.72 (0.04, 4.57) | 1.06 (0.06, 6.62) | 1.25 (0.22, 4.91) | – |
| -55 (T>C) | HBD.c.-105T>C | 1326 4 | 0.88 (0.28, 2.40) | 1.45 (0.25, 5.67) | – | 1.25 (0.22, 4.91) | – |
| CD 59 (A) | HBD.c.178delA | 1337 4 | 0.88 (0.28, 2.40) | – | – | 2.50 (0.80, 6.68) | – |
| Hb Aβ2-Wrens | HBD.c.295G>A | 1339 4 | 0.88 (0.28, 2.40) | – | – | 2.50 (0.80, 6.68) | – |
| -30 (T>C) | HBD.c.80T>C | 1329 2 | 0.44 (0.08, 1.76) | – | – | 1.25 (0.22, 4.91) | – |
| Hb Aβ2-NYU | HBD.c.39T>A | 1354 2 | 0.44 (0.08, 1.76) | 0.72 (0.04, 4.57) | – | 0.62 (0.03, 3.96) | – |
| Aβ2/6 (or Hb B2) | HBD.c.49G>C | 1356 | 1 | 0.22 (0.01, 1.42) | 0.72 (0.04, 4.57) | – | – | – |
| Hb Aβ2-Etolia | HBD.c.257T>C | 1376 | 1 | 0.22 (0.01, 1.42) | 0.72 (0.04, 4.57) | – | – | – |

Table 4. Allele frequencies in 428 carriers of δ-globin gene mutations in Cyprus and in individual districts. 

n: number of alleles.

less prevalent. In Limassol, higher frequencies of Hb Aβ2-Yokoshima and Hb Aβ2-Pelendri are observed compared to Nicosia and Larnaca/Famagusta, respectively.

**β-thalassaemia patients.** All known live 592 β-thalassaemia patients in Cyprus were genotyped at the CING and, with a population of 659115 Greek Cypriots (accounting for 98.8% of the total population), the prevalence of β-thalassaemia patients is estimated to be around 0.9 cases per 1000 people. Despite the application of a successful prevention programme, the prevalence is only slightly lower than earlier estimates of 1 in 10004, which can be mainly attributed to the better survival of β-thalassaemia patients50. In addition, it was recently reported that the prevalence of β-thalassaemia carriers has been decreasing over the past 25 years, from an estimated 15–18% to around 12% of the population5, so that the present population of β-thalassaemia patients (average age of 41 years) is representative of a previously higher carrier rate.

Table 5 shows the relative allele and genotype frequencies in β-thalassaemia patients, including frequencies for each individual district. Notably, five mutations account for more than 95% of all β-thalassaemia alleles in the patient population. As expected, the most common β-globin gene mutation is IVS 1-110 (G>A), with a percentage of 72.72%, followed by alleles IVS 1-6 (T>C) and IVS 1-1 (G>A), with frequencies of 12.42 and 5.15%, respectively. Consequently, homozygosity for IVS 1-110 (G>A) is the most common genotype in β-thalassaemia patients with a frequency of 52.7%, while 18.92% of the patients are compound heterozygous for IVS 1-110 (G>A) and IVS 1-6 (T>C), with the latter allele usually associated with a milder phenotype than IVS 1-110 G>A56. Notably, the frequency of IVS 1-110 (G>A) is lower in patients than in carriers, while the frequency of IVS 1-6 (T>C) is much higher, possibly due to a better survival rate in patients carrying the milder IVS 1-6 (T>C) mutation.

**Hb H disease patients.** For the first time, we have identified all 595 known Hb H disease patients in Cyprus and, thus, the prevalence of Hb H disease in the population is around 0.9 cases per 1000 persons. Hb H disease is caused by a combination of δ and α-thalassaemia alleles, and Table 6 shows the frequencies of δ and α-thalassaemia mutations and the observed genotype frequencies in Hb H disease patients in Cyprus and in individual districts. Two common alleles, namely -α3.7 and IVS 1-1 (-5 bp), account for more than 95% of all α-thalassaemia mutations and, similarly, deletions --MED I and -α20.5 account for more than 98% of all δ-thalassaemia mutations. Notably, the --MED I deletion and the IVS 1-1 (-5 bp) allele are particularly prevalent in the east part of the island, namely in the Larnaca/Famagusta districts, while the -α20.5 deletion is more common in Nicosia than in other districts, mirroring similar observations in the α-thalassaemia carriers dataset (Table 2). Compound heterozygosity of δ-thalassaemia mutations with the -α3.7 deletion is the most common genotype, with a frequency of 82.35%. Importantly, compound heterozygosity of --MED I or -α20.5 with the IVS 1-1 (-5 bp) α-thalassaemia mutation, a nondeletional form of Hb H disease that is often associated with a more severe phenotype, is observed in 13.11% of the patients.

**Geographic distribution of haemoglobinopathies.** Important differences are observed in the molecular spectrum of haemoglobinopathies across different districts, as discussed in previous sections, even though Cyprus is a small island with a total area of 9251 km². In addition to the district-specific molecular spectra, differences are observed in the prevalence of haemoglobinopathies in different districts, as illustrated in Fig. 1. The figure shows the fraction of the population living in different districts, according to the latest population census, compared to the fraction of carriers of α-, β- and δ-globin gene mutations in the same districts, as reflected through the datasets used in this study, thus indicating under- and over-representation of different types of
haemoglobinopathies relative to the share of the total population. Prevalence of haemoglobinopathies is generally higher than the island average in the Larnaca/Famagusta district, particularly for carriers of α- and δ-globin gene mutations, while an overrepresentation of carriers for α-globin gene mutations, only, is observed in Nicosia. In contrast, Limassol, the second-most populous district in Cyprus, has a lower prevalence of carriers of α- and δ-globin gene mutations and the same is observed in Paphos, the least populated district. As demonstrated in an earlier study for structural Hb variants, a more detailed analysis of the geographic distribution of haemoglobinopathies would provide a valuable insight into the prevalence of thalassaemia mutations in Cyprus.

**Conclusions**

This study represents the most significant update on the status of haemoglobinopathies in Cyprus for over two decades and reports the analysis of molecular data collected during 1995–2015 at the Molecular Genetics

| Mutation/Genotype | Cyprus-wide | Per district (%) |
|-------------------|-------------|------------------|
|                   | n           | Frequency (%)    | Larnaca/Famagusta | Limassol | Nicosia | Paphos |
| 3-globin mutations|             |                  |                  |          |         |        |
| IVS I-110 (G>A)   | 861         | 72.72            | 75.69            | 74.51    | 68.33   | 81.82  |
| IVS I-6 (T>C)     | 147         | 12.42            | 12.85            | 10.13    | 14.14   | 9.09   |
| IVS I-1 (G>A)     | 61          | 5.15             | 5.56             | 5.23     | 5.18    | 3.41   |
| IVS II-745 (C>G)  | 41          | 3.46             | 3.47             | 2.29     | 4.18    | 3.41   |
| CD 39 (CAG>TAG)   | 28          | 2.36             | 0.35             | 2.94     | 3.39    | 1.14   |
| Hb S              | 20          | 1.69             | 0.35             | 1.96     | 2.39    | 1.14   |
| -87 (C>G)         | 12          | 1.01             | 1.39             | 1.63     | 0.60    | -      |
| CAP +33 (C>G)     | 6           | 0.51             | -                | -        | 1.20    | -      |
| IVS II-1 (G>A)    | 4           | 0.34             | -                | 0.65     | 0.40    | -      |
| CD 44 (-C)        | 2           | 0.17             | -                | 0.65     | -       | -      |
| -86 (C>G)         | 1           | 0.08             | -                | -        | 0.20    | -      |
| CD 5 (-CT)        | 1           | 0.08             | 0.35             | -        | -       | -      |

| 3-globin genotypes|             |                  |                  |          |         |        |
|                   |             |                  |                  |          |         |        |
| IVS I-110 (G>A)  / IVS I-110 (G>A) | 312 | 52.70 | 59.03 | 52.29 | 47.41 | 63.64 |
| IVS I-110 (G>A)  / IVS I-6 (T>C)    | 112 | 18.92 | 19.44 | 16.99 | 19.92 | 18.18 |
| IVS I-110 (G>A)  / IVS II-745 (C>G)  | 27  | 4.56  | 4.17  | 4.58  | 4.38  | 6.82  |
| IVS I-110 (G>A)  / CD 39 (CAG>TAG)  | 21  | 3.55  | -     | 5.88  | 4.38  | 2.27  |
| IVS I-110 (G>A)  / Hb S              | 13  | 2.20  | 0.69  | 3.27  | 2.39  | 2.27  |
| IVS I-6 (T>C)  / IVS I-6 (T>C)       | 12  | 2.03  | 2.08  | 1.31  | 2.79  | -     |
| IVS I-110 (G>A)  / -87 (C>G)         | 9   | 1.52  | 2.08  | 3.27  | 0.40  | -     |
| IVS I-1 (G>A)  / IVS II-745 (C>G)    | 6   | 1.01  | 2.08  | -     | 1.20  | -     |
| CAP +33 (C>G)  / CD 39 (CAG>TAG)     | 4   | 0.68  | -     | -     | 1.59  | -     |
| IVS I-110 (G>A)  / IVS II-1 (G>A)    | 4   | 0.68  | -     | 1.31  | 0.80  | -     |
| IVS I-6 (T>C)  / IVS II-745 (C>G)    | 4   | 0.68  | 0.69  | -     | 1.20  | -     |
| IVS I-1 (G>A)  / IVS I-6 (T>C)       | 3   | 0.51  | 0.69  | -     | 0.80  | -     |
| Hb S/Hb S        | 2           | 0.34  | -     | -     | 0.80  | -     |
| IVS I-110 G>A  / CAP +33 (C>G)       | 2   | 0.34  | -     | -     | 0.80  | -     |
| IVS I-6 (T>C)  / Hb S                | 2   | 0.34  | -     | 0.65  | 0.40  | -     |
| -87 C>G / CD 39 (CAG>TAG)            | 1   | 0.17  | -     | -     | 0.40  | -     |
| CD 44 (-C) / IVS I-1 G>A             | 1   | 0.17  | -     | 0.65  | -     | -     |
| IVS I-1 G>A  / -87 C>G                | 1   | 0.17  | 0.69  | -     | -     | -     |
| IVS I-1 G>A  / CD 44 (-C)             | 1   | 0.17  | -     | 0.65  | -     | -     |
| IVS I-1 G>A  / IVS I-1 G>A            | 1   | 0.17  | 0.69  | -     | -     | -     |
| IVS I-110 G>A  / -86 C>G              | 1   | 0.17  | -     | -     | 0.40  | -     |
| IVS I-110 G>A  / CD 5 (-CT)           | 1   | 0.17  | 0.69  | -     | -     | -     |
| IVS I-6 (T>C)  / -87 C>G              | 1   | 0.17  | -     | -     | 0.40  | -     |
| IVS I-6 (T>C)  / CD 39 (CAG>TAG)      | 1   | 0.17  | 0.69  | -     | -     | -     |
| IVS II-745 C>G / CD 39 (CAG>TAG)      | 1   | 0.17  | -     | -     | 0.40  | -     |
| IVS II-745 C>G / Hb S                 | 1   | 0.17  | -     | -     | 0.40  | -     |
| IVS II-745 C>G / IVS II-745 C>G       | 1   | 0.17  | -     | -     | 0.40  | -     |

Table 5. Allele frequencies and genotypes in 592 β-thalassaemia patients in Cyprus and in individual districts.
Table 6. Frequencies of α-globin gene mutations and genotypes in 595 Hb H disease patients in Cyprus and in individual districts.

| Mutation/Genotype | Cyprus-wide | Per District (%)<sup>*</sup> |
|-------------------|-------------|-----------------------------|
|                   | n           | Frequency (%) | Larnaca/Famagusta | Limassol | Nicosia | Paphos |
| α<sup>+</sup> mutations |             |               |                   |          |         |        |
| IVS I-1 (-5 bp)   | 78          | 13.11         | 17.22             | 7.14     | 12.5    | 7.69   |
| Poly(A) AATAAA>AATGAA | 17         | 2.86          | 0.96              | 8.16     | 1.95    | –      |
| CD 108 (-C)       | 5           | 0.84          | 0.48              | 1.02     | 0.78    | 7.69   |
| Hb Icaria         | 3           | 0.50          | –                 | 2.04     | 0.39    | –      |
| α<sup>-</sup>4.2  | 1           | 0.17          | –                 | –        | 0.39    | –      |
| Hb Agrinio        | 1           | 0.17          | –                 | –        | 0.39    | –      |
| α<sup>-</sup> mutations |         |               |                   |          |         |        |
| --MED I           | 483         | 81.18         | 89.47             | 79.59    | 73.83   | 92.31  |
| α<sup>-</sup>20.5 | 105         | 17.65         | 10.53             | 16.33    | 25.00   | 7.69   |
| HS40 deletion     | 3           | 0.50          | –                 | 2.04     | 0.39    | –      |
| --MED II          | 2           | 0.34          | –                 | 2.04     | –       | –      |
| --SEA             | 2           | 0.34          | –                 | –        | 0.78    | –      |
| α<sup>-</sup>3.7 / --MED I | 396    | 66.55         | 73.21             | 64.29    | 61.33   | 76.92  |
| α<sup>-</sup>3.7 (type I) / α<sup>-</sup>20.5 | 88  | 14.79         | 8.13              | 14.29    | 21.09   | 7.69   |
| IVS 1-1 (-5 bp) / --MED I | 64  | 10.76         | 14.83             | 6.12     | 9.38    | 7.69   |
| IVS 1-1 (-5 bp) / α<sup>-</sup>20.5 | 14  | 2.35          | 2.39              | 1.02     | 3.12    | –      |
| Poly(A) AATAAA>AATGAA / --MED I | 13  | 2.18          | 0.96              | 6.12     | 1.17    | –      |
| CD 108 (-C) / --MED I | 5   | 0.84          | 0.48              | 1.02     | 0.78    | 7.69   |
| α<sup>-</sup>3.7 (type I) / HS40 deletion | 3  | 0.50          | –                 | 2.04     | 0.39    | –      |
| Poly(A) AATAAA>AATGAA / α<sup>-</sup>20.5 | 3  | 0.50          | –                 | 1.02     | 0.78    | –      |
| Hb Icaria / --MED I | 3   | 0.50          | –                 | 2.04     | 0.39    | –      |
| α<sup>-</sup>3.7 (type I) / --SEA | 2   | 0.34          | –                 | –        | 0.78    | –      |
| α<sup>-</sup>3.7 (type I) / --MED II | 1   | 0.17          | –                 | 1.02     | –       | –      |
| α<sup>-</sup>4.2 / --MED I | 1   | 0.17          | –                 | –        | 0.39    | –      |
| Hb Agrinio / --MED I | 1   | 0.17          | –                 | –        | 0.39    | –      |
| Poly(A) AATAAA>AATGAA / --MED II | 1  | 0.17          | –                 | 1.02     | –       | –      |

Figure 1. The distribution of carriers of α-, β- and δ-globin gene mutations per district, compared to the distribution of population in each district in Cyprus. The map of Cyprus was generated using the free web-tool Pixel Map Generator (http://pixelmap.amcharts.com) and was subsequently modified using Pixelmator v3.4.1 (http://www.pixelemator.com/mac) for OSX. The chart on the right was created using R (version 3.2.4).
Thalassaemia department at the CING, the reference laboratory for genetic analysis in Cyprus. Notably, the total sample size of around 15000 individuals (patients and carriers) used in this study is much larger than the sample sizes used in previous studies [19,21,26], making the current up-to-date report the most reliable investigation on the status and distribution of haemoglobinopathies in Cyprus. The estimated β-thalassaemia carrier rate is around 12–15%, i.e. around 130 thousands. Thus, initial datasets of 9287 and 4700 carriers of α-thalassaemia and Hb H disease patients. To our knowledge, this is the first comprehensive analysis of the national β-thalassaemia population and Hb H disease population in any country; hence, this study represents the first major step towards the challenging task to analyse and correlate genotype and phenotype for thalassaemia patients in Cyprus, which will be our future direction.

A limitation of this study is the lack of a random sample for the calculation of α-thalassaemia carrier frequencies, because a number of silent carriers are not detected by the population screening programme and, thus, are not referred to the CING for genetic analysis. Hence and because we analysed data collected through the routine molecular analysis at the CING since 1995, we could not compile a random dataset for the calculation of relative allele frequencies. The same limitation, however, is true for other studies of α-thalassaemia allele frequencies, even where this is not explicitly stated, mainly due to challenges involved in diagnosis [11,12]. A separate investigation should be performed on a random dataset to precisely determine the α-thalassaemia allele frequencies.

In addition and for the first time, we have identified and genotyped all thalassaemia patients in Cyprus, specifically β-thalassaemia and Hb H disease patients. To our knowledge, this is the first comprehensive analysis of the national β-thalassaemia population and Hb H disease population in any country; hence, this study represents the first major step towards the challenging task to analyse and correlate genotype and phenotype for thalassaemia patients in Cyprus, which will be our future direction.

References

1. Kountouris, P. et al. IhaGenes: An Interactive Database for Haemoglobin Variations and Epidemiology. PLoS ONE 9, e103020 (2014).
2. Modell, B. & Darlison, M. Global epidemiology of haemoglobin disorders and derived service indicators. Bull. World Health Organ. 86, 480–487 (2008).
3. Cappellini, M. D. et al. Guidelines for the clinical management of thalassaemia. 1–202 (Thalassaemia International Federation, 2008).
4. Angastiniotis, M. A., Kyriakidou, S. & Hadjiminas, M. How thalassaemia was controlled in Cyprus. Hemoglobin 24, 171–180 (2000).
5. Kyrri, A. R. et al. Alpha-thalassaemia in the population of Cyprus. Br. J. Haematol. 91, 83–88 (1998).
6. Angastiniotis, M. A., Kyriakidou, S. & Hadjiminas, M. Prevention of thalassaemia in Cyprus. The Lancet 1, 369–371 (1981).
7. Kyriacou, K. & Piel, F. B. & Weatherall, D. J. The α-thalassemias. N Engl J Med 371, 1908–1916 (2014).
8. Old, J. et al. Prevention of Thalassaemias and Other Haemoglobinopathies. 1, (Thalassaemia International Federation, 2013).
9. Chui, D. H. K. & Galanello, R. Hemoglobin : α- and β-globin gene mutations in Greek cypriots.
10. Tüzmen, S., Basak, A. N. & Baysal, E. Rare β-thalassaemia variants in the Turkish Cypriot population. Hemoglobin 33, 81–94 (2009).
11. Lederer, C. W. et al. Triplicated alpha-globin loci in humans. Proceedings of the National Academy of Sciences 77, 518–521 (1980).
12. Cao, A. & Galanello, R. Beta-thalassaemia. Genet Med 13, 83–88 (2011).
13. Piel, F. B. & Weatherall, D. J. The α-thalassemias. N Engl J Med 371, 1908–1916 (2014).
14. Telfer, P. et al. Beta-thalassemia. The Lancet 336, 834–837 (1990).
15. Kyrri, A. R. et al. Hemoglobin Variants in Cyprus.
16. Coo, A. et al. Triplicated alpha-globin loci in humans. Proceedings of the National Academy of Sciences 77, 518–521 (1980).
17. Baysal, E. et al. β-Thalassaemia in the population of Cyprus. Br. J. Haematol. 81, 607–609 (1992).
18. Pavlou, E. et al. Analysis of delta-globin gene mutations in Greek cypriots. Blood 82, 1647–1651 (1993).
19. Pavlou, E. et al. β-Thalassaemia in Cyprus. Hemoglobin 30, 455–462 (2006).
20. Tüzmen, S., Basak, A. N. & Baysal, E. Rare β-thalassaemia mutation IVS-II-848 (C>A) first reported in a Turkish cypriot family. Ann. J. Hematol. 54, 338–339 (1997).
21. Lederer, C. W. et al. Hb Agrinio [alpha29(B10)Le–>uPro (alpha2)] in combination with (~MED I). Results in a severe form of Hb H disease. Hemoglobin 32, 237–246 (2008).
22. Kyrri, A. R. et al. Hemoglobin Variants in Cyprus. Hemoglobin 33, 81–94 (2009).
23. Lederer, C. et al. α- and β-globin chain variant [HBDc.c.60C>A] in four families with diverse globin genotypes. Annals of Hematology 93, 1625–1627 (2014).
24. Old, J. M., Varawalla, N. Y. & Weatherall, D. J. Rapid detection and prenatal diagnosis of beta-thalassaemia: studies in Indian and Cypriot populations in the UK. The Lancet 336, 834–837 (1990).
25. Traeger-Synodinos, J., Maragoudaki, E., Vrettou, C., Kanavakis, E. & Kattamis, C. Rare beta-thalassemia alleles in the Greek and Greek Cypriot populations. Hemoglobin 22, 89–94 (1998).
26. Telfer, P. et al. Survival of medically treated thalassaemia patients in Cyprus. Trends and risk factors over the period 1980–2004. Haematologica 91, 1187–1192 (2006).

Author Contributions

PK, analysed the data and wrote the manuscript with I.K.; P.K. and I.K. assembled the data; P.K., I.K., M.K. and S.C. conceived the study; A.R.K., E.Kal., C.M., C.I., L.K., G.H., N.A. and M.A. took part in the population screening programme for the identification of carriers; I.K., T.P., G.C., E.P., E.Kar., X.F., M.Pe., M.Ph., P.F., C.W.L. and M.K. took part in the genetic analysis of carriers and patients at the CING; S.C., E.P., I.S., M.H., M.S., A.K. and M.A. contributed to the data collection and identification of β-thalassaemia and Hb H disease patients. All authors have read and approved the final manuscript.
Additional Information

Competing financial interests: The authors declare no competing financial interests.

How to cite this article: Kountouris, P. et al. The molecular spectrum and distribution of haemoglobinopathies in Cyprus: a 20-year retrospective study. Sci. Rep. 6, 26371; doi: 10.1038/srep26371 (2016).

This work is licensed under a Creative Commons Attribution 4.0 International License. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/