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Population Screening for Hemoglobinopathies

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Abstract

Hemoglobinopathies are the most common single-gene disorders in the world. Their prevalence is predicted to increase in the future, and low-income hemoglobinopathy-endemic regions need to manage most of the world's affected persons. International organizations, governments, and other stakeholders have initiated national or regional prevention programs in both endemic and nonendemic countries by performing population screening for α- and β-thalassemia, HbE disease, and sickle cell disease in neonates, adolescents, reproductive-age adults (preconceptionally or in the early antenatal period), and family members of diagnosed cases. The main aim of screening is to reduce the number of affected births and, in the case of sickle cell disease, reduce childhood morbidity and mortality. Screening strategies vary depending on the population group, but a few common screening test methods are universally used. We discuss the salient features of population-screening programs around the globe as well as current and proposed screening test methodologies.

INTRODUCTION

Hemoglobinopathies, which result from quantitative defects in hemoglobin (Hb) (the thalassemias) and Hb structural variants, are prevalent in malaria-endemic regions (the Mediterranean, Asia, and sub-Saharan Africa) owing to natural selection (144). They have also become increasingly prevalent in nonendemic Europe, North America, and Australia owing to population migrations and have therefore become a global health problem. Urgent measures are needed to curtail the disease burden and the associated negative economic impact, which affects mainly low-income countries (160). The clinically significant hemoglobinopathies include α- and β-thalassemia, sickle cell disease (SCD), HbE disease, and HbC disease (100, 105). It is estimated that more than 330,000 affected births occur annually, mainly with SCD (83%) and the thalassemias (17%) (100). In 2008, the World Health Organization (WHO) reported that 5.2% of the world's population carry a significant hemoglobinopathy-causing genomic variant and that 24.0% are carriers of all known hemoglobinopathy-causing genomic variants (both pathogenic and nonpathogenic) (100). Phenotype–genotype correlations generated using data from the 1000 Genomes Project have shown that there can be a chance of hemoglobinopathy occurring in 14 per 10,000 persons in the next generation (31).

In the past, hemoglobinopathies were a neglected cause of morbidity and mortality. Acknowledging the lack of suitable recognition of these disorders and considering other factors that help to sustain these disorders, in 2006 the WHO (167, p. 7; 168, p. 26) passed two resolutions urging its member states to "design, implement and reinforce in a systematic, equitable and effective manner, comprehensive national, integrated programmes for the prevention and management" of thalassemia and other hemoglobinopathies, including SCD, with the participation of all stakeholders involved in hemoglobinopathy diagnosis, hospital and community management, and research. This goal was further endorsed at the WHO and Thalassaemia International Federation meeting in November 2007 (169). The Thalassaemia International Federation has been playing an important advocacy role in promoting population screening for hemoglobinopathies, and details of its work have been published (138). In 2014, a European expert group, after studying the impact of hemoglobinopathies in Europe, published 10 policy recommendations for the region, including increasing awareness of and screening for hemoglobinopathies (94).

According to the WHO, the aim of hemoglobinopathy screening is to identify carriers in order to assess the risk of producing severely affected progeny, followed by genetic counseling and the provision of options to avoid such births, ideally prior to conception (56, 166). The main factors determining the selection of a particular hemoglobinopathy-screening strategy are the prevalence of hemoglobinopathy, genetic variability, and cost effectiveness. Where prevalence is high, mass screening strategies can be adopted that target people at different life stages, such as adolescents (e.g., screening of schoolchildren) and reproductive-age females and males or male partners of carrier females (premarital, preconceptional, or antenatal screening). In regions with low prevalence or where consanguinity is common, screening of family members of index cases (cascade screening) is recommended. Newborn screening (NBS) is performed for SCD to prevent morbidity and mortality associated with the disease. The success of a screening program will depend on the target population complying with the proposed objectives of the program, and therefore programs should plan for widespread public awareness campaigns; take into consideration cultural, social, and ethical aspects of the population; provide detailed and timely counseling to at-risk individuals or couples; and offer prenatal diagnosis (PND) (63).

There are now population-screening programs for hemoglobinopathies in all continents of the globe (43) (**Figure 1**). In this article, we discuss the basis of the screening procedures and then review reported hemoglobinopathy-screening programs in both endemic and nonendemic

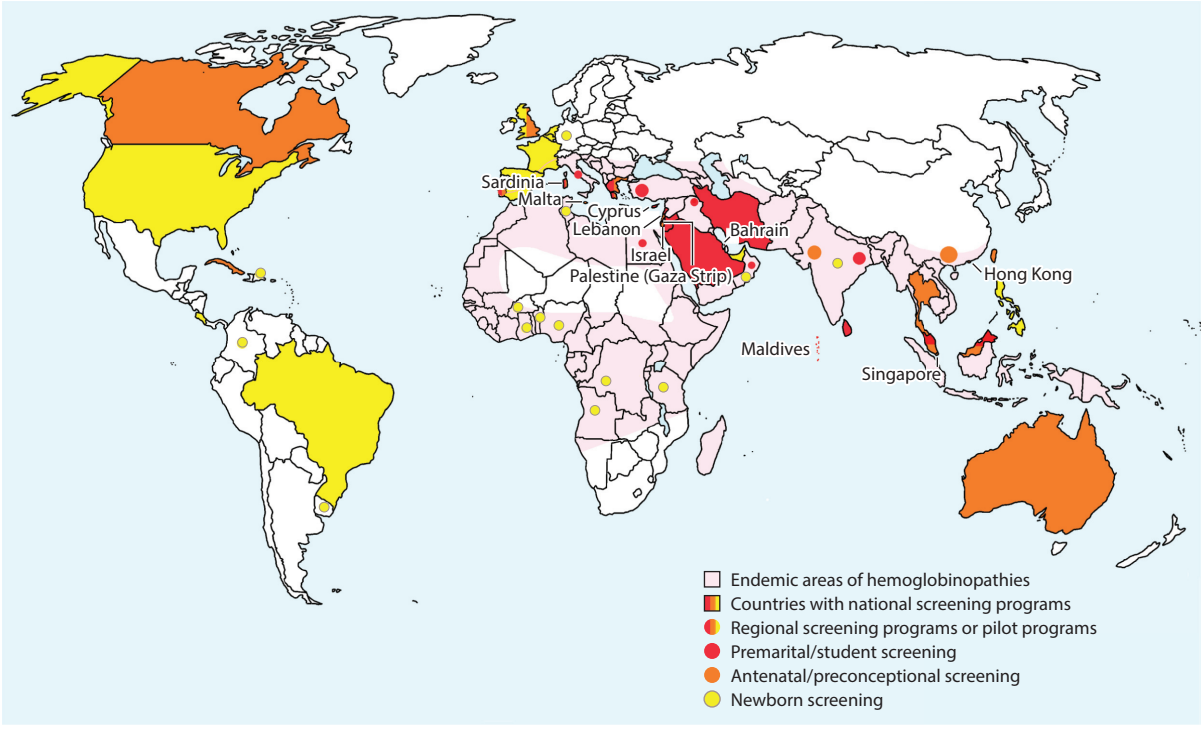

Figure 1

Hemoglobinopathy-screening programs worldwide. Country coloring indicates where national hemoglobinopathy-screening programs are in place, and small colored circles indicate where regional screening programs or pilot programs are in place; the specific colors indicate the main focus of each program.

regions, covering their unique features, challenges, outcomes, and future plans for improvement. We conclude by giving a brief glimpse into novel screening technologies that have been recently published, which may lead to a paradigm shift in future strategies for population screening for hemoglobinopathies.

POPULATION SCREENING FOR HEMOGLOBINOPATHIES: THE BASIS AND RATIONALE FOR SCREENING PROCEDURES

The Hb molecule is a heterotetramer formed by two unlike pairs of globin protein chains. Each globin chain contains an oxygen-binding heme group in the center that stores and carries oxygen to tissues (170). The globin proteins are coded by α- and β-globin gene clusters (**Supplemental Figure 1*a***). The α-globin gene cluster, comprising the Hb subunit alpha A1 gene (*HBA1*/α_1) and Hb subunit alpha A2 gene (*HBA2*/α_2), is located in the telomeric region on the short arm of human chromosome 16, specifically at 16p13.3. Additionally, the α-globin gene cluster contains the embryonic ζ_2 and θ genes along with three pseudogenes ($\Psi\zeta_1$, $\Psi\alpha_1$, and $\Psi\alpha_2$). The α_1- and α_2-globin genes are similar in their coding regions, and each consists of three exons and two introns. However, these two genes differ in their expression, with the α_2-globin gene yielding nearly two to three times as much of its encoded protein compared with the α1-globin gene. The expression of the α-globin genes is regulated by four highly conserved noncoding sequences

Supplemental Material >

known as multispecies conserved sequences R1–R4 (MSC-R1–R4), located 25–65 kb upstream of the α-globin genes. MCS-R2, also known as HS-40, which lies (along with MCS-R1 and MCS-R3) within the introns of the widely expressed *NPRL3* gene, is a crucial remote regulatory element of α-globin gene expression. MSC-R1–R4 interact with promoter sequences (TATA box and CCAAT box) of the α-globin genes to regulate the transcription of these genes (64, 76).

The β-globin proteins are encoded by the Hb subunit beta gene (*HBB*) located on human chromosome 11p15.4 in a gene cluster that consists of four other globin genes—the embryonic ε-globin gene, fetal Gγ-globin gene, fetal Aγ-globin gene, and δ-globin gene—and a pseudogene (**Supplemental Figure 1*b***). The *HBB* gene spans approximately 1.6 kb and has three exons and two introns, similar to the α-globin genes, but with a considerably larger intron 2 (IVS2). The major regulatory region of this gene cluster, known as the locus control region, is located 50 kb upstream from the *HBB* gene and consists of four erythroid-specific DNase hypersensitive sites (HS-1–4). Transcription regulation of the *HBB* gene occurs via the interaction of the locus control region with promoter sequences, including TATA, CCAAT, and CACCC boxes, which are the binding sites for transcription factors such as erythroid Kruppel-like factor 1 and GATA-1 (27, 146).

Supplemental Material >

During human development, sequential switching of Hb types occurs from embryonic to fetal to adult type by coordinated on-and-off switching of gene expression at the α-globin (*HBA1* and *HBA2*) and *HBB* gene clusters. The first switch from embryonic to fetal Hb (HbF), which is formed by two α-globin proteins combining with two γ-globin proteins ($\alpha_2\gamma_2$), occurs within the first trimester of pregnancy. The second, from HbF to adult Hb, occurs around birth, with the downregulation of γ-globin gene expression and marked upregulation in the expression of β-globin genes as well as δ-globin genes. The adult Hb phenotype is fully established by the end of the first year of life and consists of HbA ($\alpha_2\beta_2$, 97%), HbA2 ($\alpha_2\delta_2$, 2%), and HbF ($\alpha_2\gamma_2$, 1%) (134).

Genetic defects affecting the rate of globin chain production lead to α- or β-thalassemia depending on the type of globin chain affected, giving rise to the most common monogenic disorders in the world (163). α-Thalassemia commonly results from one or more deletions in the α-globin gene region or, more rarely, from point mutations (**Supplemental Table 1**), and β-thalassemia commonly results from point mutations in the β-globin gene (**Supplemental Figure 2**, **Supplemental Table 2**). The HbVar database includes hundreds of genomic variants that give rise to both α- and β-thalassemia (65). Structural Hb variants arise mostly from single amino-acid substitutions in the *HBB* gene, and more than 1,000 variants have been reported in the HbVar database (65), but only a few occur in sufficiently large proportions in individual populations to warrant their testing in population screening. Such variants include sickle Hb (HbS) and HbE disease (100). HbS in a heterozygous, homozygous, or compound heterozygous state that gives rise to red blood cell sickling is known as SCD.

In the thalassemias, globin protein chain imbalance is minimized by a combination of available excess globin protein chains. In α-thalassemia, during fetal life, γ-globin protein tetramers (γ_4, Hb Barts) are formed, and after birth, β-globin protein tetramers (β_4, HbH) are formed. In β-thalassemia, compensatory increases of HbA-2 ($\alpha_2\delta_2$) and HbF ($\alpha_2\gamma_2$) occur. These changes can be detected using qualitative tests or quantitative tests, including acid and alkaline Hb electrophoresis, capillary electrophoresis (CE), isoelectric focusing (IEF), and high-performance liquid chromatography (HPLC). These tests are used alone or in combination to identify carriers or affected individuals. HbS in the deoxygenated state causes insoluble polymers inside red blood cells, leading to sickling red blood cell deformity, which can be detected in a peripheral blood smear or by carrying out a simple, non-technically-demanding rapid screening test or sickle solubility test; HbS can also be detected using the above-mentioned tests (18, 33, 109, 110).

The clinical phenotype in hemoglobinopathies is determined by the underlying genotype, and the phenotype can vary from silent carrier states to mild, moderate, or severe anemia, which can be

lethal (151, 158). The thalassemias typically give rise to hypochromic microcytic red cell indices, and therefore a complete blood count (CBC) is the primary screen for hemoglobinopathies; cutoffs of a mean cell volume value of <79 fL and a mean cell Hb concentration value of <27 pg are used to identify carriers. However, the mean cell volume and mean cell Hb may not be abnormal in milder forms of β-thalassemias, compound heterozygous states for α- and β-thalassemia, and silent α-thalassemia traits (163). Also, clinically important *HBB* variants (HbS and HbC) cannot be detected by measuring mean cell volume and mean cell Hb and require a different approach for screening, including red blood cell sickling tests to detect SCD, staining of red blood cells to detect HbH inclusions (precipitated β-globin chains) for α-thalassemia, the use of the qualitative or quantitative methods mentioned above to detect Hb variants, and/or direct DNA testing, the last of which is required to confirm α-thalassemia (18, 37, 151).

Despite their genetic heterogeneity, β-thalassemia carriers have a relatively consistent range of elevated HbA2 levels, which is therefore a reliable marker for screening and diagnosis of the condition (84). The HbA2 level can be falsely normal with milder *HBB* gene variants causing β^+-thalassemia, δ-thalassemia, and coinheritance of β^+- and δ-thalassemia (146) and in the presence of severe iron deficiency anemia (28). Iron deficiency is the most common cause of anemia in the developing world (86) and needs to be considered in the differential diagnosis of anemia and low mean cell volume and mean cell Hb in screening programs. Owing to the developmental physiology of Hb types, elevation of HbA2 does not occur at birth, and β-thalassemia carriers cannot be diagnosed using HbA2 levels in NBS programs. However, structural Hb variants can be detected at birth, and therefore neonatal screening is carried out mainly to diagnose SCD (66). The naked-eye single-tube red cell osmotic fragility test (NESTROFT) can identify microcytic red blood cells, which have reduced osmotic fragility, as in β-thalassemia, and the dichlorophenolindophenol (DCIP) dye test can identify unstable Hb, such as the variant HbE. Both NESTROFT and DCIP dye tests are simple, low-cost tests that can replace the relatively more expensive CBC frequently used for screening, and Asian screening programs have used these tests to screen for β-thalassemia and HbE disease (62, 153).

The thalassemias are found in the equatorial belt from the Mediterranean region through the Middle East and India to Southeast Asia; however, population migration has resulted in the occurrence of this condition spreading all over the globe. HbS is endemic in Africa and parts of the Middle East and India. Population migration had resulted in its spread to North America, Europe, and the Caribbean region. The HbE variant is found in the Indian subcontinent and Southeast Asia, and compound heterozygosity for HbE and β-thalassemia contributes to the highest disease burden among all hemoglobinopathies. HbC, once endemic to West Africa, is also seen more widely across the world owing to population migration (121). The reported mutation spectrum of the β-globin genes is relatively narrow and unique to a given endemic population (**Supplemental Table 2**); thus, setting up population screening and (especially) genomic screening is less complicated and allows targeted screening of populations (163). However, nonendemic regions, which receive a regular influx of populations from endemic regions, require careful assessment of the spectrum of hemoglobinopathy-causing mutations in each country before any implementation of preventive measures (94).

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HEMOGLOBINOPATHY POPULATION-SCREENING PROGRAMS IN THE WORLD

The Thalassaemia International Federation has described four critical elements for a hemoglobinopathy population-screening program: the education of all stakeholders, the use of standardized methods for carrier screening, the availability of genetic counseling and genetic

diagnostic services, and PND. Furthermore, it proposes to maintain registries that can catalog the outcomes of screening programs; determine the appropriate treatments, prevention policies, health services, and resource allocation; and guide research related to hemoglobinopathies (120). The WHO and other organizations recommend the practice of voluntary carrier screening and highlight the importance of informed choice in population screening (56, 166). The majority of screening programs involve voluntary screening. However, premarital screening is mandatory in the Maldives, Middle Eastern countries, and Cyprus, although decisions regarding marriage and conception are left to individual couples (28, 43). NBS programs in countries across all continents of the globe include nationwide or target-population screening for hemoglobinopathies, mainly for SCD, along with other inherited conditions. NBS programs around the world were comprehensively reviewed in 2015 (148).

Screening for hemoglobinopathies in thalassemia-endemic regions first began in the Mediterranean basin in the latter part of the twentieth century. Greece and Cyprus started their thalassemia prevention programs in the early 1970s with premarital, preconceptional, or antenatal screening, which was voluntary in Greece (93) but mandatory or quasi-mandatory in Cyprus, depending on ethnicity (15). In Italy, Sardinia began its thalassemia prevention program in 1975 with premarital and antenatal screening (26). The first countries to implement universal thalassemia screening in Asia were the Maldives in 1992 (155) and Taiwan in 1993 (36). In the Middle East, Israel began performing targeted thalassemia screening of at-risk ethnicities in 1980 (175). By the first decade of the twenty-first century, a majority of Middle Eastern countries had implemented nationwide mandatory premarital thalassemia-screening programs (43). In Europe, the United Kingdom launched its universal voluntary NBS program in 2004 (107). Regional voluntary screening programs have also been implemented for schoolchildren in the Marseille region of France (90), among high-risk couples in the state of Virginia in the United States (112), among at-risk ethnic groups in Canada (87), and as a part of antenatal screening in Mumbai, India (43).

Screening programs have now been established on all continents, in both hemoglobinopathy-endemic and hemoglobinopathy-nonendemic regions (**Figure 1**), targeting either entire national populations or high-risk groups, and screening guidelines have been published (129). Below, we review the core features of population-screening programs in both hemoglobinopathy-endemic regions (the Mediterranean region, the Middle East, Asia, Africa, and Latin America) and hemoglobinopathy-nonendemic regions (Europe, North America, and Australia) (**Table 1**).

Screening Programs in Endemic Regions

This section presents detailed descriptions of the activities carried out by all stakeholders involved in hemoglobinopathy screening, along with the aims and outcomes of the population-screening programs, in countries located in the Mediterranean region, the Middle East, Asia, Africa, and Latin America.

The Mediterranean region. Prospective carrier screening, as a mode of prevention of thalassemia major, was considered for the first time by the countries in the Mediterranean region (e.g., Greece, Sardinia, and Cyprus), where consanguinity was a significant cause of childhood morbidity and mortality (15, 26, 93). The thalassemia prevention program in Sardinia began with hospital- and community-based voluntary screening of carriers, counseling, and antenatal screening of fetal blood (26). Twenty years after the introduction of the program, affected births had been reduced (to 1 in 4,000). Furthermore, the identification of common mutations causing β-thalassemia has enabled the establishment of phenotype–genotype correlations and antenatal

Table 1 Hemoglobinopathy-screening programs around the world

Country	Year(s) of implementation	Program type(s)	Focus group(s)[a]	Mandatory?	Screening method(s)	Reference(s)
Endemic countries						
Mediterranean Europe						
Cyprus	1973	Nationwide	Premarital, preconceptional, and antenatal	Yes	CBC, BP, HE	15, 44
Greece	1974	Nationwide	Premarital and antenatal	No	CBC, HE/HPLC, sickling test	93
Italy	1975	Regional (Sardinia)	Premarital and antenatal	No	CBC, HPLC	26, 28–30
	1975	Regional (Latium)	Secondary school pupils	No	HPLC	14
France	1978–1985	Regional (Marseille)	Secondary school pupils	No	CBC, IEF, HE/HPLC	90
	2000	Nationwide/targeted (based on ethnicity)	Neonatal (SCD)	No	—	19
Malta	1991	Nationwide	Antenatal	No	—	58
Portugal	1990s	Regional (southern districts)	Premarital and antenatal	No	CBC, HPLC	95
Spain	2003	Nationwide/targeted (based on ethnicity)	Neonatal (SCD)	—	HPLC	119
Middle East and West Asia						
Israel	1980	Targeted (at-risk populations)	Preconceptional and antenatal	No	—	175
Bahrain	1992	Nationwide	Antenatal	—	—	3
	1998	Nationwide	Secondary school pupils	—	—	3
	2007	Nationwide	Neonatal	No	HPLC, IEF	6
Lebanon	1994	Nationwide	Premarital	Yes	CBC, HE	1
Iran	1997	Nationwide	Premarital	Yes	CBC, HPLC	133
Oman	1999	Regional (8 hospitals)	Premarital	No	CBC, sickling test, HE, HPLC	128
	2005–2007	Pilot (2 hospitals)	Neonatal	No	CBC, sickling test, HE, HPLC	12
Palestine	2000	Regional (Gaza Strip)	Premarital	Yes	CBC, HE	143
Turkey	2003	Regional (33 provinces)	Premarital	No	—	25
Saudi Arabia	2004	Nationwide	Premarital	Yes	CBC, HPLC	9
Jordan	2004	Nationwide	Premarital	Yes	CBC, HPLC	71
United Arab Emirates	2005	Nationwide	Neonatal	No	HPLC	4
Iraq	2008	Regional (Kurdistan)	Premarital	Yes	CBC, HPLC	5

(*Continued*)

Table 1 (*Continued*)

Country	Year(s) of implementation	Program type(s)	Focus group(s)[a]	Mandatory?	Screening method(s)	Reference(s)
South Asia						
Maldives	1992	Nationwide	Premarital	Yes (since 2012)	CBC, HPLC	59, 155
Sri Lanka	2006	Nationwide	Premarital (over age 15)	No	CBC, HPLC	60, 103
India	1997–2003	Regional (Mumbai, Maharashtra)	Antenatal	No	OFT, CBC, HPLC	40
	—	Regional (Surat, Gujarat)	Antenatal	No	CBC, HE, HPLC	22
	2007–2009	Regional (Indore, Madhya Pradesh)	Antenatal	No	CBC, HPLC	20
	2003–2007	Regional (Uttar Pradesh)	Premarital	No	CBC, HPLC	140
	1999–2011	Regional (West Bengal)	Population	No	CBC, HPLC	35
	2012–2015	Regional (Hooghly, West Bengal)	Population	No	CBC, HPLC	21
	2004–2008	Regional (Gujarat)	Population	No	CBC, HPLC	116
	2009–2011	Regional (1 center, Nagpur, Maharashtra)	Neonatal (SCD)	No	Solubility test, HPLC	83
Southeast Asia						
Thailand	1997	Nationwide	Antenatal	No	OFT, CBC, HE/HPLC	60
Singapore	1997	Nationwide	Antenatal	No	CBC, HE	141
Malaysia	2004	Nationwide	Premarital, antenatal, schoolchildren, and cascade	No	CBC, HE/HPLC	99
Philippines	2014	Nationwide	Neonatal	—	—	49
East Asia						
Southern China	1993–2003	Regional (Guangzhou)	Antenatal	No	CBC, HPLC	91
	2010	Regional (Guangxi)	Preconceptional	No	CBC, HE/HPLC	60
	2000	Regional (Hong Kong)	Antenatal	No	CBC, HbH inclusions, HPLC	77
Taiwan	1993	Nationwide	Antenatal	No	CBC, HPLC	36
Africa						
Egypt	1993–2005	Regional	Schoolchildren	No	—	55
Republic of Benin	1993	Pilot (Cotonou)	Neonatal (SCD)	No	IEF	127

(*Continued*)

Table 1 (*Continued*)

Country	Year(s) of implementation	Program type(s)	Focus group(s)[a]	Mandatory?	Screening method(s)	Reference(s)
Tunisia	—	Pilot (Tunis and northwestern region)	Neonatal	No	—	57
Ghana	1995–2004	Pilot (Kumasi)	Neonatal (SCD)	No	IEF	114
Burkina Faso	2000–2004	Pilot (Ouagadougou)	Neonatal (SCD)	No	IEF	85
Nigeria	2000	Pilot (Benin City)	Neonatal (SCD)	No	IEF	113
Democratic Republic of the Congo	—	Pilot (Kinshasa and four provinces)	Neonatal (SCD)	No	IEF	152
Angola	2011–2013	Pilot (Luanda)	Neonatal (SCD)	Yes	IEF	96
Tanzania	2015–2016	Pilot (Dar es Salaam)	Neonatal (SCD)	No	IEF	149
Latin America						
Cuba	1983	Nationwide	Antenatal	No	—	2
Brazil	2001	Nationwide	Neonatal (SCD)	Yes	IEF, HPLC	23, 89
Costa Rica	—	Nationwide	Neonatal	Yes	HPLC	23
Colombia	2000–2014	Regional (eight cities)	Neonatal	No	HE	53
Uruguay	2013	Pilot	Neonatal (SCD)	—	HPLC	126
Dominican Republic	2014	On request	Neonatal	No	—	126
Nonendemic, developed countries						
United States	2005	Nationwide	Neonatal	Yes	HPLC/IEF	20a, 147, 157
Canada	—	Nationwide/targeted (based on ethnicity)	Preconceptional and antenatal	No	CBC, HE/HPLC	87
United Kingdom	2004	Nationwide	Antenatal and neonatal	No	CBC, HPLC/IEF	107, 125
Netherlands	2007	Nationwide	Neonatal	No	HPLC	24, 74
Belgium	1994	Regional (Brussels)	Neonatal	—	IEF, HPLC	69
	2002	Regional (Liège)	Neonatal	—	IEF, HPLC	69
Germany	—	Pilot	Neonatal	—	MS/MS	67
Australia	—	Regional (varies among states)	Preconceptional and antenatal	No	CBC, HE/HPLC	45, 142

Abbreviations: BP, blood picture; CBC, complete blood count; HE, hemoglobin electrophoresis; HPLC, high-performance liquid chromatography; IEF, isoelectric focusing; MS/MS, tandem mass spectrometry; OFT, osmotic fragility test, SCD, sickle cell disease; —, information not available.

[a]Population screening includes premarital, preconceptional, and antenatal screening; screening of schoolchildren; and screening of the family members of affected individuals.

DNA diagnosis (29). The thalassemia prevention program in Cyprus was similar to that in Sardinia and also resulted in a significant reduction in affected births (15, 44). Both thalassemia and SCD were prevalent in Greece, and the population-screening programs had to include the multiethnic migrant populations originating from hemoglobinopathy-endemic regions of the south, which required special training of health-care workers; the heterogeneous molecular spectrum of the disease also posed a challenge for screening (93). The Greek thalassemia and SCD prevention program was supported by the government in collaboration with blood transfusion services. As in the programs of other countries in the region, it offered voluntary screening following community education. PND was offered centrally in Athens. After three decades, births affected by thalassemia major, HbH disease, and SCD combined were reduced to 15 per year from a previously predicted count of 120–130 per year (93).

The success of the thalassemia prevention programs in the Mediterranean region was due mainly to massive public education campaigns via mass media, posters, information booklets, training of health-care workers, incorporation of thalassemia education into school curricula, and extended family screening (28, 30). In a region of France within the Mediterranean basin, including Marseille, a port city in the south, carrier screening of children in high secondary school was conducted between 1978 and 1985. A follow-up of this cohort 14 years later showed that screening followed by counseling had had a positive influence on the prevention of affected births (90). Mainland France currently has a national NBS and antenatal screening program that is carried out on request (94). Spain, which has the lowest prevalence of thalassemia in the region, has seen increases in both thalassemia and SCD (heterogeneously distributed) following immigration, mainly from Africa and China. Spain offers national NBS to at-risk ethnicities, and premarital and prenatal screening is performed on demand (119). Spain also created a national hemoglobinopathy registry in 2014, which includes data on thalassemia major, thalassemia intermedia, and SCD patients (32). Malta, an archipelago in the central Mediterranean Sea, started its national thalassemia-screening program in 1991 by offering antenatal screening, and the positive effects of screening and counseling were evident 15 years later in the reduction of affected births (58). Portugal's β-thalassemia-screening program commenced in the 1990s at government health centers in several districts of the country. This program provides a "carrier card" showing the diagnosis (β-thalassemia or sickle cell carrier state) to those who are screened, along with counseling and PND (95).

The Middle East. In the Arab-speaking world, which comprises 22 countries, the carrier rates for hemoglobinopathies vary widely among countries: The ranges of reported rates for α- and β-thalassemia are 1–58% and 1–11%, respectively, and the range of the reported rate for SCD is 0.3–30%. The mutation spectrum of both α- and β-globin genes has been reviewed previously (70). Recessive disorders, including hemoglobinopathies, are maintained by the prevalence of consanguineous marriages in the region, which can be as high as 60% (8). Following the implementation of a thalassemia-screening program targeted to at-risk populations in Israel in 1980 (175), population screening in the Middle East has gradually increased. These programs focus mainly on premarital screening. NBS and antenatal screening are also carried out in some countries (**Table 1**). The countries or regions with mandatory premarital screening and genetic counseling programs are Lebanon (1), Iran (133), the Gaza Strip (143), Saudi Arabia (9), Jordan (71), and the Kurdistan region in northern Iraq (5). One exception is Turkey, where the national thalassemia-screening program offers voluntary premarital screening to couples in 33 provinces in the Mediterranean region and southern Turkey, where hemoglobinopathies are prevalent. Despite the voluntary nature of the programs, there was increasing participation (30% in 2003 and 81% in 2008), resulting in a 90% reduction in the number of affected births (25).

The aim of these premarital screening and genetic counseling programs is to reduce the prevalence of hemoglobinopathies by preventing at-risk marriages and performing PND followed by termination of the affected fetuses, which is offered in some countries (130). Policies on medical termination of affected pregnancies differ between countries: The laws in Iran and Iraq allow medical termination (5, 133), while those of Lebanon and the United Arab Emirates do not (1, 7). Despite high uptake of premarital carrier screening, sociocultural factors hinder the significant reduction of at-risk marriages, but termination of affected fetuses has reduced the rate of affected births. The influencing factors, legal issues, and outcomes of the premarital screening and genetic counseling programs in the Middle East have been reviewed (13, 130). Preimplantation genetic diagnosis, which is accepted by the Islamic religion, was first introduced in Saudi Arabia, and so far it has been considered a more favorable option than termination of affected fetuses (70). Although it is more expensive and time-consuming and requires technical expertise, it might be a component in future hemoglobinopathy-screening programs in the region (8).

In the Middle East, NBS is carried out for hemoglobinopathies (148). In the United Arab Emirates, the NBS program, including screening for hemoglobinopathies (primarily SCD), has been implemented nationwide since 2005 (4). In Egypt, both β-thalassemia and SCD occur, with the former being more prevalent, but screening is not performed at a national level. A screening program for schoolchildren from different regions was conducted from 1993 to 2005, and antenatal diagnosis and PND for at-risk pregnancies are performed on request in certain centers (55). Egypt has had poor uptake of PND, but following in-depth nondirected counseling, which included alleviating religious misconceptions, opinions about PND and pregnancy termination became more favorable (54). Thalassemia and SCD education and screening of schoolchildren and university students have been carried out in certain regions in the Middle East (70). Increased awareness among adolescents and young adults is expected to facilitate better uptake of preventive measures prior to marriage decisions (11).

Asia. The countries in the Indian subcontinent, Southeast and East Asian countries, Taiwan, and China's southern region are endemic for α- and β-thalassemia and *HBB* gene variants, mainly HbE disease. The Southeast and East Asian countries in particular are endemic for Hb Constant Spring and Hb Barts hydrops fetalis. Hemoglobinopathies are rarely seen in Korea and Japan, and the main mutation spectrums in these countries have been described (61, 62). The island nations of the Maldives and Taiwan commenced universal hemoglobinopathy-screening programs in 1992 and 1993, respectively, and national thalassemia control programs have been established in Sri Lanka, Thailand, Malaysia, Singapore, and the Philippines (43). **Table 1** summarizes the details of these programs. In the Philippines, the national NBS program has included screening for hemoglobinopathies since 2014 (49); other countries in the region do not yet include hemoglobinopathies in their NBS programs (148).

Maldivian Blood Services and a nongovernmental organization, the Society for Health Education, jointly started offering nationwide screening for thalassemia, genetic counseling, and education campaigns in the Maldives. To ensure the further success of the program, the Maldives enacted a thalassemia prevention law in 2012 that imposed compulsory screening of citizens by 18 years of age; furthermore, following the consent of religious leaders, abortion for severe fetal malformations was legalized, initiating the establishment of PND (59, 155). A national thalassemia registry was established in 1993 and has recorded a falling prevalence of carriers and affected births, but the affected numbers are still significant for a small nation, and studies have shown that the main factor for this is a lack of awareness (59).

Taiwan's national thalassemia-screening program, which targets pregnant females, has been successful in reducing the carrier prevalence and affected births. Screening begins with a CBC

during the booking visit at an antenatal clinic; a low mean cell volume and mean cell Hb leads to tests of the serum ferritin for iron deficiency, measurement of HbA2 and HbF levels, DNA testing, PND, and ultimately, if necessary, termination of the pregnancy. DNA testing requires that the couple make a subsidized payment (36). Taiwan's screening program has been challenging because of interracial marriages following migrations from surrounding endemic regions that changed the mutation spectrum (156), and significant numbers of affected births continue to occur. Plans are under way to strengthen genetic counseling services in the country. Creating awareness and early screening that targets high school students have been considered better modes of thalassemia prevention compared with antenatal screening, as affected births continue to occur (36).

Thailand established its national prevention and control program for thalassemia in 1997 under the auspices of the government's Departments of Medical Services, Health, and Medical Sciences, with the aim of preventing and controlling severe forms of α- and β-thalassemia. A national policy for thalassemia was published in 2005. The program is targeted at pregnant females and their partners, free of charge, and voluntary. It includes health education of all stakeholders, national annual meetings on thalassemia, the establishment of cost-effective diagnostic protocols, low-cost screening tests (NESTROFT and DCIP dye tests), and PND (60). The problems encountered in the current Thai control program are late initiation of screening, poor uptake, and the absence of the partner for testing. To overcome these issues, premarital testing and testing of all females are being considered. However, the screening program has successfully reduced the number of births of affected newborns over the years (171).

In 2004, the Malaysian Ministry of Health initiated a national thalassemia prevention and control program. The program includes all of the components described by the Thalassaemia International Federation for a screening program, including PND and establishment of a thalassemia registry (99). The program performs cascade screening in all cases of thalassemia major and targeted screening of adolescents (through school screening programs) and pregnant females. Details of the Malaysian thalassemia-screening protocol published by the Ministry of Health are available online (98).

Routine nationwide voluntary screening for thalassemia in Singapore was introduced in 1997. The program targets women at their antenatal booking visit and consists of a CBC test followed by a confirmatory DNA test. It has also initiated a thalassemia registry. The success of the program has been reflected in a reduction of the number of affected births, from 15–20 per year to 1 per year by 2003 (141).

Hong Kong's thalassemia antenatal screening program began at the start of the twenty-first century. Screening is carried out by an initial CBC followed by testing of the partner and confirmatory testing using standard methods; PND is performed if needed, with the aim of carrying it out prior to 18 weeks of gestation. The program guidelines were published by the Hong Kong College of Obstetricians and Gynaecologists in 2003 (77).

In the Philippines, both α- and β-thalassemia predominate, although HbE disease also occurs. Population screening has been performed using HPLC, and the country has good genetic diagnostic and counseling services. NBS is performed by both the government and private sectors, and hemoglobinopathy screening was incorporated into the expanded NBS panel in 2014. By 2016, the program had screened more than 50,000 babies, detecting mainly HbH disease and α-thalassemia, along with one case of HbE disease (49, 115).

Sri Lanka's government funded a national thalassemia control program that launched in 2006 with the creation of regional thalassemia-screening centers (60). Voluntary screening is offered to persons aged 15 years or above. Cascade screening is carried out in parents and siblings of children with thalassemia major; the screening involves a CBC and a mean cell volume measurement, followed by Hb electrophoresis, HPLC, or DNA testing (103, 132). Pregnancy termination

is illegal in Sri Lanka except when the life of the mother is at risk, and since termination of thalassemia-affected pregnancies is not an option, the theme of the prevention program is "safe marriages"—avoiding marriages between two carriers, thus preventing affected pregnancies via screening of adolescents and premarital adults. A person who is not a carrier is given a green card, whereas a carrier is given a pink card (103). Although antenatal screening and PND are not offered in the national program, a recent 10-year study of PND carried out for the pregnancies of couples at risk for thalassemia has been published, which highlights the availability of DNA testing for PND in the country (106). With the aim of guiding national policy with accurate data collection pertaining to thalassemia management and prevention, a thalassemia registry was established (118) that includes patient demographics, treatment data, and treatment costs. Detailed understanding of the distribution of the hemoglobinopathy-causing genetic variants in a given population (genomic micromapping) enables better planning of preventive strategies (159). Genomic micromapping studies conducted in Sri Lanka show the heterogeneous distribution of the mutations in the country (48). Studies in a Sri Lankan province have shown that people have a good opinion of screening programs (102), but lack of awareness has led to poor usage (103).

Screening for hemoglobinopathies has been performed in different states in India, targeting schoolchildren, college and university students, antenatal mothers, and high-risk tribal, ethnic, and caste groups, includes cascade screening (**Table 1**). The CBC, measurement of HbA2 levels, HPLC, DNA testing, and PND have been used for screening and diagnosis (39). The main hemoglobinopathies in India are β-thalassemia, Hb variants (HbE and HbD), and SCD, which show a heterogeneous distribution within the large country. The mutation spectrum is wide, but a few common mutations account for the majority of β-thalassemia cases (38, 153) (**Supplemental Table 2**). Both governmental and nongovernmental organizations have been conducting public awareness, screening, and genetic counseling programs aiming to reduce the disease burden throughout India for almost half a decade. The states of West Bengal and Gujarat have well-established thalassemia control programs that conduct screening and education, keep electronic records of patient data, and carry out PND (39). Screening for SCD is performed in endemic regions, such as the state of Chhattisgarh (117). NBS for SCD is also carried out in some centers, and the use of dried blood spots has enable screening of home deliveries (136). As in other endemic regions, stigmatization, which leads to poor usage or nondisclosure of carrier status at the time of marriage, has been reported in India, but it has a greater negative impact on screening and prevention because India has a large carrier population. Therefore, screening prior to conception and early antenatal screening followed by PND and cascade screening have been identified as more suitable screening methods for India than screening prior to marriage (153). Genomic micromapping studies have been conducted in Maharashtra, Gujarat, and Surat, targeting students, reproductive-age adults, and specific community groups. More widespread micromapping studies and development of a thalassemia database are recommended for India, as these measures can help determine the exact disease burden of hemoglobinopathies in order to enable the development of more effective screening strategies (46, 159). The current status of hemoglobinopathies in India has been reviewed, and pathways for developing effective control measures, including the need for a national thalassemia control program in India, were highlighted recently (39).

Supplemental Material >

Bangladesh, Pakistan, and Nepal are three developing countries in the Indian subcontinent, and national screening or prevention programs have not been reported in these countries. In a recent review, Hossain et al. (80) provided insight into factors that affect hemoglobinopathy screening in Bangladesh, which has more than 160 million people. Both β-thalassemia and HbE disease are prevalent in this country. The primary factors affecting screening are the limited access of rural populations to screening facilities and a poor literacy rate, which have hampered public awareness campaigns. Health professionals and grade school, college, and university students

have been identified as the groups to target for awareness campaigns. Cascade screening has been recognized as the best economically viable strategy for screening; premarital screening has been considered ineffective because of the problem of stigmatization.

β-Thalassemia is the most common hemoglobinopathy in Pakistan, and recognizing the need to identify carriers and enable PND, Ansari et al. (17) studied the mutational spectrum in different ethnicities in Pakistan and devised a three-tiered screening algorithm based on DNA testing that can be applied globally to screen populations originating from Pakistan. The main factor that influences population screening in Pakistan is the lack of health-care infrastructure to support large-scale screening. As consanguineous marriages are prevalent, cascade screening has been performed to detect carriers. A national screening program that can assess the true disease burden in the country and plan preventive strategies to reduce affected thalassemia births in Pakistan has been proposed (10).

The most common hemoglobinopathies in China are α- and β-thalassemia, which have a heterogeneous distribution throughout the country (174). Mandatory premarital screening for inherited disorders, including hemoglobinopathies, was practiced in China between 1992 and 2003 (13). Voluntary hemoglobinopathy-screening programs continue to be carried out at regional and provincial levels in China, and reports of successful programs that offer preconceptional or prenatal screening followed by PND have been published (60, 91). More recent reports of novel screening strategies have emerged from China. In the Hunan province of southern China, antenatal mothers have been screened using a gene chip that detects *HBA* and *HBB* gene mutations common to the Chinese population (72). These comprehensive population-specific genetic data are contributed to a data bank, which the Chinese Center for Disease Control and Prevention is accumulating to develop future screening strategies in regions of the country where thalassemia is prevalent. A simple, cost-effective screening tool has been developed that can use red blood cell indices to differentiate β-thalassemia carriers from individuals with iron deficiency anemia (150). Hemoglobinopathy screening using more expensive next-generation sequencing (NGS) technology in 10,111 couples from five provinces in southern China demonstrated the detection of carrier states that were not detected by CBC screening or HPLC (137).

Africa. In the first report of its kind, evidence-based analysis of the global burden of SCD done by Piel et al. (122) indicated that the disease is most prevalent in sub-Saharan Africa. There are more than 3 million carriers (64.4% of annual sickle carrier births in the world) and more than 200,000 affected individuals (75.5% of the annual affected births in the world). SCD is most prevalent in the Senegal, Benin, and Bantu regions. National screening and preventive programs have not been implemented in any African countries, but pilot projects for NBS for hemoglobinopathies have begun in Angola (96), Nigeria (113), Ghana (114), the Democratic Republic of the Congo (152), and the Republic of Benin (127). Published reports of SCD screening and preventive programs in the African region were included in a 2015 review of SCD among children in Africa (104).

In Uganda, using the country's national early infant diagnosis program, Ndeezi et al. (111) estimated the prevalence of infants with SCD between February 2014 and March 2015; the results of the study are expected to help develop a national strategy for NBS. In Tanzania, a two-year pilot project that began in 2015 helped to develop national guidelines for SCD, and work to develop a national NBS program and SCD registry is ongoing (149). Factors associated with the implementation of NBS programs in Uganda and Tanzania, two countries with a high burden of SCD, have been discussed (68).

NBS along with screening during extended vaccination programs has been suggested as a way to ensure greater surveillance of SCD in Africa (51). The sickle solubility test is the most widely used screening method in Africa. The high cost of maintenance and need for trained staff have

limited the use of HPLC or IEF to private institutions and tertiary-care government hospitals. The development of infrastructure with foreign collaborations and/or the incorporation of simple, inexpensive point-of-care testing methods can overcome economic factors and enable screening of populations living far away from screening facilities (97). Furthermore, as first-cousin marriages are common, premarital screening and genetic counseling, supplemented by the incorporation of education into the school curriculum and the creation of awareness among early teens, have been identified as primary preventive measures to be implemented (51).

Latin America. Latin America consists of nearly 20 countries, including Mexico in North America as well as Central and South American countries. SCD is prevalent in many countries in the region, which has been attributed mainly to the history of forced migration of Africans to Latin American countries through the slave trade. Population migrations continue to occur mainly from the African region, and the number of SCD carriers and affected individuals is expected to increase further owing to a lack of comprehensive screening services in most of the countries of the region (123). A majority of countries, including Cuba, Costa Rica, Chile, Uruguay, Brazil, Mexico, Argentina, Colombia, Paraguay, and Venezuela, have established nationwide NBS programs, with a few countries (Cuba, Costa Rica, Chile, and Uruguay) achieving nearly 100% coverage and Brazil reaching over 80% coverage. However, only Costa Rica and Brazil currently perform nationwide mandatory NBS (23).

Among the countries in Latin America, Brazil has the largest African American population and the largest population estimates for SCD, with nearly 30,000 cases and approximately 1 in 1,000 newborns estimated to be affected with SCD (82). In 2001, the national NBS program was expanded to include SCD. The centralized NBS program is coordinated by the Ministry of Health and includes five main elements. First, all newborns undergo laboratory testing. Second, following these initial screening tests, an active search for suspected cases and their families is performed. Third, confirmatory tests are performed to diagnose the disease. Fourth, IEF and HPLC are used for hemoglobinopathy screening and diagnosis. And fifth, the treatment of diagnosed cases is offered at the national level, and follow-up by a multidisciplinary team is arranged when needed (89). Brazil's NBS program demonstrated the importance of universal NBS for hemoglobinopathies and the need for organized treatment and preventive strategies in improving the survival and quality of life of affected children, which has been demonstrated by the reduction of SCD-related mortality after the program was implemented (47). Other than Brazil, Costa Rica is the only country in the region with a nationwide mandatory NBS program for hemoglobinopathies. The existing NBS program screens for 29 conditions, including mandatory screening for α- and β-thalassemia and SCD (23).

A few other Latin American countries are implementing hemoglobinopathy screening as a component of the existing NBS programs and have conducted pilot studies prior to nationwide implementation. In Colombia, there are reports of several independent NBS programs for hemoglobinopathies that use gel electrophoresis for Hb analysis. From 2000 to 2014, these programs screened more than 27,000 newborns; they reported that abnormal Hb was present in 1.3% of newborns screened, of which HbS accounted for 43% (53). In 2013, Uruguay began a pilot NBS program for hemoglobinopathies that uses HPLC, with the verification of the positive cases performed using IEF. The Dominican Republic also began its national NBS program as recently as 2014 and offers hemoglobinopathy screening on request. However, nationwide hemoglobinopathy-screening programs are yet to be established in these countries (126). Cuba has an established nationwide NBS program that has achieved nearly 100% coverage, but screening for hemoglobinopathies is not yet included (23). The Cuban national program for SCD prevention focuses on antenatal screening, PND, and prenatal counseling (2).

Screening Programs in Developed, Nonendemic Regions

This section describes the hemoglobinopathy-screening strategies adopted in European and North American countries and Australia.

Europe. Following population migrations from endemic regions, hemoglobinopathies now occur in all regions of Europe. SCD is more common than the thalassemias (101). The northern European countries—Austria, Belgium, Denmark, France, Germany, the Netherlands, Sweden, and the United Kingdom—have had a large influx of immigrants from endemic regions, with national carrier rates for hemoglobinopathies ranging from 0.34% to 0.75% (16). A 2012 survey estimated that approximately 44,000 patients with hemoglobinopathies live in 10 European Union countries: Belgium, Cyprus, France, Germany, Greece, Italy, the Netherlands, Spain, Sweden, and the United Kingdom. Given the growing problem, implementing hemoglobinopathy-screening programs has been considered a priority (94).

The world's first linked antenatal and neonatal hemoglobinopathy-screening program was implemented in the United Kingdom in 2004, and the hope is that this successful program will become a model for hemoglobinopathy-screening programs in other countries (107). It offers screening to all pregnant women, prospective fathers of pregnant carriers, and all newborn babies as a part of a newborn blood spot screening program. The fundamental principles are to carry out antenatal screening by 10 weeks of gestation and to carry out 50% of the PND before 12 weeks and 6 days, allowing sufficient time for couples to make informed choices regarding whether to terminate the pregnancy (125). The initial key performance indicators were coverage, timeliness of testing (i.e., testing by 10 weeks' gestation), completion of a family origin questionnaire, and timeliness of report availability; these indicators were subsequently updated in 2017 (42). Details regarding the program coverage for 2015–2016 have been published (34). Overall, the UK hemoglobinopathy-screening program covers all aspects stated by the Thalassaemia International Federation, and its policies, including standardized testing methods, have been published; the methods used for screening include CBC, HPLC, CE, and IEF (109, 110). Successes of the UK antenatal screening and NBS programs have also been published (52, 139).

Mainland France has been receiving a heavy influx of emigrant populations from hemoglobinopathy-endemic regions, mainly African (16). France's national SCD NBS program began in 2000 for at-risk babies, and although screening is not universally practiced, the program has shown good coverage and uptake (19).

Following recommendations from the Health Council of the Netherlands, the Dutch government added screening for hemoglobinopathies to the existing NBS program in January 2007; the screening was aimed primarily at SCD, but owing to their importance, β-thalassemia major, all the clinically relevant *HBB* gene variants (HbE, HbD, and HbC), and Hb Barts were also included. Revised recommendations for neonatal screening were published in 2015 (74). Key features of the Dutch NBS program include an opt-out option that allows the parents to not learn the results of testing and a provision for not reporting incidental findings of other hemoglobinopathy variants.

In 2007, the Health Council of the Netherlands recommended preconception testing for couples (73). Implementing a universal prenatal screening program had been a subject of much debate (145). NBS has also been available in Belgium since 1994 (69), and the effect of screening in reducing infant morbidity and mortality was recently reported (88). Population migration has resulted in the introduction of hemoglobinopathies in Germany. Although pilot studies on NBS for SCD have been carried out, Germany has not yet integrated SCD screening into its NBS program (67). Similarly, thalassemia and SCD are emerging diseases in Sweden, but screening programs for hemoglobinopathies have not yet been implemented (75).

European countries that maintain national hemoglobinopathy registries for β-thalassemia and/or SCD include France (124), Greece (154), Italy (41), the United Kingdom (108), Belgium, Cyprus, the Netherlands, and Malta (94). The registries contain epidemiological, clinical, and treatment data; details on the availability of health services; and so on. The UK and French registries can be accessed online.

North America. In the United States, the state of Virginia began carrying out voluntary premarital screening for high-risk SCD couples in 1970 as part of the Virginia Sickle Cell Anemia Awareness Program. This program—the first recorded population-screening program in the world—included genotyping, counseling, and follow-up with couples (112). As in other Western nations, migration from hemoglobinopathy-endemic areas has led to an increased prevalence of SCD and thalassemia in the United States (78), which requires education of health-care providers and the public; implementation of screening when CBC abnormalities are detected, including cascade screening; and PND along with genetic counseling (135). In 1975, the state of New York added NBS as part of its newborn blood spot screening programs for SCD, and following recommendations by the American College of Medical Genetics, the state established universal mandatory screening in 2006 (20a, 147, 157). This program was primarily intended to screen for SCD, but screening for thalassemia is carried out if warranted based on CBC findings, as directed by primary care physicians or pediatric units, which also gather ethnicity data and perform parental screening in the process of confirming the diagnosis (78). Primary screening for hemoglobinopathies is usually performed by CBC followed by IEF or HPLC to confirm the diagnosis; alternatively, either electrophoresis or DNA studies are performed. Ethnically diverse regions, such as California, often perform mandatory screening and follow-up for other hemoglobinopathies, including HbH disease and HbE β-thalassemia (33, 79).

In Canada, the Society of Obstetricians and Gynaecologists of Canada Genetics Committee and the Canadian College of Medical Geneticists make joint recommendations for carrier screening of reproductive-age adults; these recommendations are regularly updated and revised, with the last revision published in 2016 (165). Carrier screening is carried out on persons originating from hemoglobinopathy-endemic regions using CBC, HPLC, electrophoresis, HbF and HbA2 quantification, serum ferritin, and HbH testing. Screening of partners follows a positive screening test, and genetic counseling is carried out for high-risk couples. PND is offered at any time during pregnancy, preferably in the first trimester. As reported in 2010, NBS for hemoglobinopathies was being carried out regionally with varying practices, and the development of a national NBS program was under consideration (164).

Australia. As in Europe and North America, migration from endemic regions has led to an increased prevalence of hemoglobinopathies in Australia, including SCD and β-thalassemia. A national policy for screening for the disorders has yet to be implemented, and regional practices vary (45, 142). However, an Australian hemoglobinopathy registry has been established. This registry collects clinical data from patients with thalassemia and SCD (available at **https://www.monash.edu/medicine/sphpm/registries/hbr**) and could lead to policy changes in the country (45).

CONCLUSIONS AND FUTURE DIRECTIONS

Our review shows that, in all regions of the world where hemoglobinopathies occur in significant proportions of the population, measures to control the disease burden have been implemented as part of either national or regional programs. Reduction in the prevalence of affected births has been used as the measure to assess the success of many programs. The evidence indicates

that factors that influence population screening (13, 43) differ among population groups, and therefore a universal screening strategy cannot be adopted in all populations; instead, specific population-based screening strategies need to be developed by relevant governments and/or other stakeholders.

The types of investigations used to detect the main forms of α- and β-thalassemia and structural variants, HbE disease, and SCD were discussed above. The screening programs use similar protocols for screening and diagnosis of hemoglobinopathies. Simple, cost-effective alternatives for CBC have been used in Asia. However, if economic factors are eliminated, the recommended methodologies published in various sets of guidelines (33, 109, 110, 151) could be universally practiced.

The success of screening programs depends greatly on acceptance by the population to be screened, which relies on creating good awareness in the target population and the availability of genetic counseling following diagnosis (28, 30). This review also highlights that lack of awareness and lack of easy access to diagnostic and counseling services, especially for rural communities in Asia and Africa. Effective counseling can create positive attitudes and/or dispel incorrect beliefs, leading to better uptake and good outcomes (54). Other factors that affect acceptance of a screening program include literacy rate and educational status, which may be more relevant to populations in and originating from hemoglobinopathy-endemic regions (80). Obligatory screening ensures that a greater number of people are screened but does not necessarily ensure the success of a prevention program, as shown by the screening programs in Middle Eastern countries (130). Micromapping studies have shown that the mutation spectrum is more varied in populations living in small geographical zones, and therefore the current known prevalence rates for hemoglobinopathies (162) are underestimates (159). The availability of more micromapping data will contribute to better planning of future population-screening programs (161).

New screening methods are urgently needed that can differentiate carrier states from disease states and rule out common conditions, such as iron deficiency anemia; are economical and simple to carry out (testing using a blood drop requires little in terms of technical expertise and instrumentation); and can enable testing in rural communities. A cost-effective method that can differentiate β-thalassemia from iron deficiency anemia has been reported from China, which has potential for hemoglobinopathy screening in Asia (150, 172). More recently, a molecular test kit that uses blood spots and can be used as a first-line screening tool for SCD, HbC disease, and neonatal screening has had good results in Africa; it does not require DNA extraction, eliminating the need for expensive equipment, and therefore could be ideal for screening in rural African communities (50). In Taiwan, other methods, such as denaturing HPLC and microarray analysis, which enable greater precision in β-thalassemia carrier detection, have been viewed as potential screening techniques (36).

This review also described the expansion of currently available technologies into hemoglobinopathy screening. These technologies include gene chips carrying population-specific mutations (72) and NGS (137), which has several advantages, including the ability to screen large cohorts and the ability to detect carrier states that are not picked up by other methods.

Tandem mass spectrometry is currently used to detect structural Hb variants (mainly SCD) in NBS programs on dried blood spots. The extension of this technology to include screening for β-thalassemia has been described in a Chinese study and offers new possibilities for NBS programs in the rest of Asia (173). As NGS technologies become more widely available, they may be incorporated into NBS programs; the issues and challenges of doing so have been discussed elsewhere (81).

In fetal medicine, preimplantation diagnosis is an area of exploration, and the recent development of a simpler testing method has been reported (131). Furthermore, owing to technical and

biological advances, noninvasive PND using fetal cells derived from maternal blood is expected to be more widely available in the next decade (92). Validation and incorporation of these new technologies into hemoglobinopathy testing guidelines could transform future hemoglobinopathy population-screening practices.

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