Newborn Screening for Sickle Cell Disease: Jamaican Experience

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ABSTRACT

Objectives: *To review the history of newborn screening for sickle cell disease with especial reference to Jamaica.*

Methods: *A summary was done of the history, the development of associated laboratory technology and the implementation of newborn screening for sickle cell disease in Jamaica.*

Results: *Screening was initiated at Victoria Jubilee Hospital, Kingston from 1973–1981, reactivated in 1995 and extended to the University Hospital of the West Indies in 1997 and to Spanish Town Hospital in 1998. From August 2008, there was a progressive recruitment of 12 hospitals in the south and west of Jamaica which has raised the frequency of islandwide newborn coverage from 25% in 1973 to 81%. The results of this extended programme in southwest Jamaica are presented. Dried blood spots collected from the umbilical cord proved stable, cheap and efficient; mean sample collection rates were 98%, maternal contamination occurred in < 1% and caused diagnostic confusion in < 0.1%. By March 31, 2015, a total of 54 566 births have been screened, detecting 161 with homozygous sickle cell (SS) disease, 125 with sickle cell-haemoglobin C (SC) disease and 36 with sickle cell-beta thalassaemia. Of the 327 babies with clinically significant sickle cell syndromes, all except five who died within seven days of birth were confirmed by four to six weeks and recruited to local sickle cell clinics.*

Conclusion: *Early detection of sickle cell disease and recruitment to clinics is known to reduce its morbidity and mortality. The methods currently detailed provide an effective and economic model of newborn screening which may be of value elsewhere.*

Keywords: Caribbean, Jamaica, newborn screening, sickle cell disease

Tamización Neonatal en la Detección de la Enfermedad de Células Falciformes: Una Experiencia Jamaicana

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RESUMEN

Objetivos: *Revisar la historia del tamizaje de recién nacidos para detectar la enfermedad de células falciformes, con especial referencia a Jamaica.*

Métodos: *Se realizó un resumen de la historia, el desarrollo de las técnicas de laboratorio asociadas, y la implementación del tamizaje neonatal para la detección de la enfermedad de células falciformes en Jamaica.*

Resultados: *La tamización se inició en el hospital "Victoria Jubilee" de Kingston, en el año 1973, terminando este período inicial en 1981. Fue luego reactivada en 1995 y extendida al Hospital Universitario de West Indies en 1997 y al Hospital de Spanish Town en 1998. A partir de agosto de 2008, se produjo una incorporación progresiva de 12 hospitales al sur y al oeste de Jamaica, elevándose la frecuencia de la cobertura de recién nacidos en toda la isla, de un 25% en 1973 a un 81%. Se presentan los resultados del programa extendido en el sudoeste de Jamaica. Las gotas de sangre seca del cordón umbilical*

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resultaron un medio de detección estable, barato y eficiente. Las tasas promedio de recogida de muestras alcanzaron un 98%, en tanto que la contaminación materna ocurrió en < 1% y causó confusión de diagnóstico en < 0.1%. Para el 31 de marzo de 2015, se habían cribado un total de 54 566 nacimientos, detectándose 161 neonatos con la enfermedad de células falciformes homocigóticas (SS), 125 con la enfermedad de células falciformes-hemoglobina C (SC), y 36 con anemia falciforme-beta talasemia. A todos los 327 bebés con síndromes de células falciformes clínicamente significativos – excepto a cinco que murieron dentro de los siete días del nacimiento – les fue confirmada la enfermedad dentro de cuatro a seis semanas, y se les remitió a clínicas locales de células falciformes.

Conclusión: *Se sabe que la detección temprana de la enfermedad de células falciformes y la remisión a las clínicas, reducen su morbilidad y mortalidad. Los métodos actualmente detallados proporcionan un modelo eficaz y económico de tamización neonatal que puede ser de valor en otros lugares.*

Palabras claves: Caribe, Jamaica, tamización neonatal, enfermedad de células falciformes

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INTRODUCTION

Sickle haemoglobin (HbS) results from a mutation of the beta globin gene with valine replacing glutamic acid at position 6 of the beta globin chain and is designated $\alpha_2\beta^s{}_2$. This substitution changes the electrophoretic mobility of the molecule but, more importantly, changes its function so that when deoxygenated, adjacent molecules tend to polymerize, raising the intracellular viscosity and ultimately deforming the red blood cells into the characteristic sickle shape. Sickle cell disease refers to a spectrum of conditions following the inheritance of HbS from both parents or from one parent along with another interacting abnormal haemoglobin from the other parent. Differential diagnosis of these conditions requires the analysis of beta chains which, although detectable in early fetal life, only account for 20–30% of adult haemoglobin at full term delivery. At birth, the presence of HbS and the absence of HbA is consistent with homozygous sickle cell (SS) disease, sickle cell-β^o thalassaemia, sickle cell-hereditary persistence of fetal haemoglobin (S-HPFH), or of double heterozygotes for HbS and another variant travelling in the position of HbS. The presence of HbS with small amounts of HbA may indicate one of the forms of sickle cell-β^+ thalassaemia and HbS with a greater amount of HbA represents the sickle cell trait. Distinction of these conditions depends upon the available technology.

The development of newborn screening

Phenylketonuria (PKU) was the first condition detectable by newborn screening in 1963 (1) and tests became available for maple syrup urine disease and classic galactosaemia in 1970, and for congenital hypothyroidism in 1974. The development of tandem mass spectrometry in the 1990s greatly expanded the range of metabolic conditions which could be detected at birth including inborn errors of metabolism of amino acids, organic acids, fatty acids and other multisystem diseases, and most states in the United States of America (USA) currently screen for 30–50 conditions. Despite the conclusions of a Consensus Development Conference at the National Institutes of Health, Bethesda, Maryland, in 1987 (2) that "the benefits of screening for sickle cell disease are so compelling that universal screening should be provided", such screening was not implemented throughout the USA until 2006 (3). Most European countries screen for a limited number of congenital conditions and screening for sickle cell disease became universal in England from 2005 (4); targeted screening has been increasingly practised in France (5), Germany (6, 7), Spain (8, 9), Italy (10), the Netherlands (11) and Belgium (12), and pilot programmes are reported from Brazil (13), India (14, 15) and several African countries (16–20). Most Caribbean countries have not yet developed screening programmes for congenital and metabolic disorders and newborn screening for sickle cell disease is inconsistent without any clear policies throughout the region. Exceptions include Guadeloupe which has practised universal screening from 1984 (21), and Jamaica which commenced programmes in 1973 but still does not offer universal screening.

NEWBORN SCREENING FOR SICKLE CELL DISEASE

Technologies for separating haemoglobin bands

For newborn diagnosis, small amounts of HbA and HbS must be identified in the presence of large amounts of HbF, and the relatively insensitive technology in 1948 led Watson *et al* (22) to state, "there is at present no certain method for predicting whether he [a newborn] will later develop [sickle cell] disease". The advent of agar gel electrophoresis at pH 6.2 in 1957 (23) allowed separation of HbA and HbS from HbF and early studies differentiated AS and SS patterns in 1000 samples from the Democratic Republic of Congo in 1969 (24) and detected six cases of SS disease among 756 babies at Yale-New Haven Hospital in 1974 (25). The technology further improved with the use of cellulose acetate membranes under alkali conditions (pH 8.4–8.9) with analysis of abnormal bands on agar gel at pH 6.2. These combined methods allowed extensive population surveys (26) and the screening to establish the Jamaican Cohort Study of Sickle Cell Disease (27, 28). Cellulose acetate pro-vides high definition but is expensive and agar gels are cheap when produced in the laboratory, but the electrophoretic characteristics of the agar may vary between batches. In the last 30 years, greater resolution of individual protein bands has been

possible with iso-electric focusing (IEF), cation exchange high performance liquid chromatography (HPLC), capillary electrophoresis (CE), mass spectrometry (MS) and tandem mass spectrometry (MS/MS). Each has advantages and disadvantages but the techniques most widely used in screening for sickle cell disease are IEF or HPLC, the former being labour intensive and the latter convenient, relatively expensive at a reagent cost of US$0.70 per sample but the available automation saves on technician time. The most recent automated Variant nbs (Bio-Rad Laboratories, Hercules, California) allows the loading of up to 384 samples completing a sample analysis with the short sickle programme every three minutes. The increasing sophistication and resolution of these technologies from the early agar gel methods to HPLC are illustrated in Fig. 1.

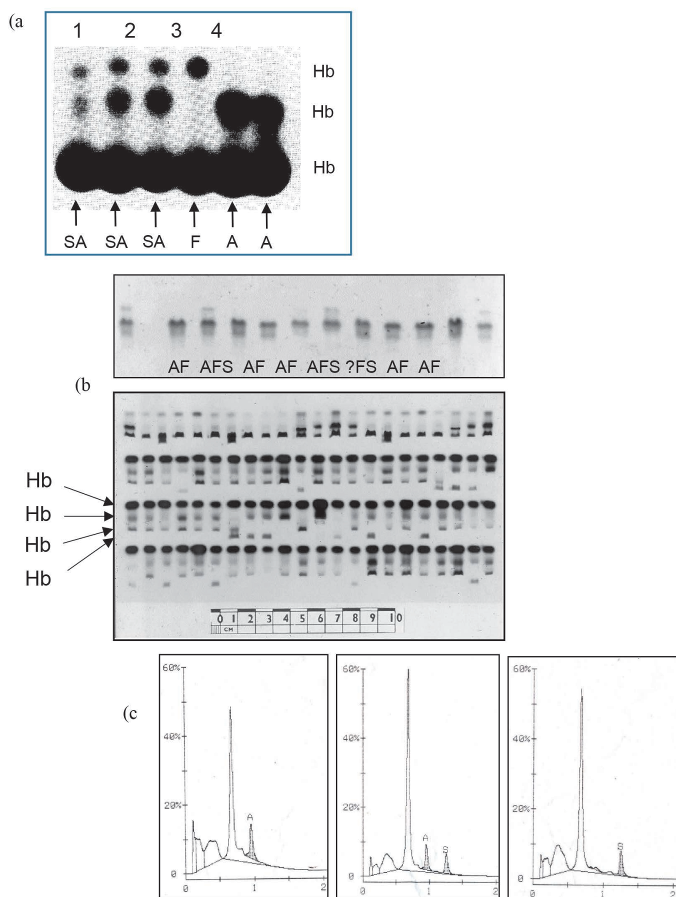

Fig. 1: Development of newborn screening methods.

(a) Agar gel electrophoresis (adapted from reference 24).
(b) Diagnostic procedures used in the Jamaican Cohort Study (27, 28); cellulose acetate electrophoresis (upper panel) and agar gel (lower panel). The agar gel has adult bloods (top row) and newborns (bottom 3 rows). All electrophoretic abnormalities on cellulose acetate electrophoresis were analysed by agar gel.
(c) Analysis of cord blood by high pressure liquid chromatography. In each panel, the major peak is HbF; reading from left, i) peaks of HbF and HbA (normal haemoglobin phenotype), ii) peaks of HbF, HbA and HbS (sickle cell trait), iii) peaks of HbF and HbS (consistent with SS disease).

Timing of the blood sample for screening

The timing of the blood sample depends on the purpose of screening since several conditions may not become apparent until after 24 hours. For example, in PKU, the elevated levels of phenylalanine only develop after the baby has been drinking milk for at least 24 hours so, in communities susceptible to PKU, blood samples are usually collected at least 24 hours after birth before discharge from hospital. Diagnosis of sickle cell disease is simpler since the gene products can be detected at birth, allowing the blood sample to be taken from the umbilical cord or by heel-prick. Cord blood samples have the advantage of ease of collection, improving the collection rates, but heel-pricks require a separate procedure which must be carried out before discharge which is often within 24 hours of delivery. A potential disadvantage of cord blood is that samples may be contaminated by the mother's blood but in practice, this only occurred in 0.8%, similar to elsewhere (29) and less than 0.1% of babies required recall for potential diagnostic confusion.

Nature of the blood sample for screening

Initially, liquid blood was collected in sodium citrate, centrifuged and the supernatant removed, before lysing red cells with saponin and then applying the haemolysate with wicks to the agar gel. Haemolysate production was later simplified by the tetrasodium EDTA method of Schneider (30) but liquid blood was prone to infection and sample deterioration. Dried blood spots (Fig. 2), pioneered by Dr Robert Guthrie of Buffalo, New York, had the advantage of simplicity, stability and easy portability. In the laboratory, a small piece of the dried blood spot is eluted in water and is then available for analysis. 'Guthrie' cards consist of a piece of filter paper attached to a clerical section requesting mother's name, infants' date of birth and contact details. Cards may be readily and cheaply produced locally with a printed section pasted to a 50 mm × 30 mm piece of #3MM chromatography paper, enabling the production of 14 000 cards from a box of 100 sheets of chromatography #3MM paper measuring 460 mm × 570 mm.

Newborn Screening for Sickle Cell Disease
Ministry of Health/Sickle Cell Trust

Babies birth date ____________ M/F Tel ______________

Mother ______________ ______________
Surname First name

Address ______________________________

Mother's birth date ____________ Del bk # ____________

Fig. 2: Typical Guthrie card used in Newborn Screening.

Significance of early diagnosis

In SS disease, symptoms commonly commence by three to four months and the greatest mortality occurs in the second six months of life; two common causes being overwhelming blood infections especially with *Streptococcus pneumoniae* and sudden enlargement of the spleen trapping the babies' blood (acute splenic sequestration). Pneumococcal prophylaxis reported initially in Jamaica (31) and later in the USA (32) showed that most infections could be prevented. Furthermore, teaching parents to examine for the spleen regularly and prophylactic splenectomy in recurrent cases reduced the death rate from acute splenic sequestration by 90% (33). These observations led the Consensus Development Conference in 1987 to justify newborn screening, a recommendation further strengthened by subsequent developments including the use of transcranial Doppler in detecting children at risk of first stroke (34), use of hydroxyurea in severely affected children (35), and the improved management of the disease from parental education and specialized clinics.

History of screening in Jamaica

Following the pilot studies in the Congo and Yale-New Haven, the first extensive use of newborn screening occurred in Jamaica where, in collaboration with Dr Leslie Williams, senior obstetrician at Victoria Jubilee Hospital (the main Government maternity hospital), 100 000 deliveries were screened between June 1973 and December 1981 (27, 28). This hospital delivered 15 000 babies at that time or 25% of the estimated 60 000 all-island deliveries. Following establishment of a representative sample of patients for the Jamaican Cohort Study of Sickle Cell Disease, newborn screening in Jamaica ceased for 14 years until September 1995 when the screening programme was reactivated. Conducted by the staff of the Medical Research Council Laboratories (Sickle Cell Unit), The University of the West Indies, with financial support from the Ministry of Health, screening recommenced on November 1, 1995, extended to the University Hospital of the West Indies from October 1997 and Spanish Town Hospital from April 1998, together covering 15 000 deliveries or approximately 30% of the estimated 50 000 all-island deliveries in 1998.

The next stimulus for expanding the newborn screening came with the development of the Manchester Project which, against the background of school students requesting knowledge of their haemoglobin genotypes, offered screening to the 5th and 6th forms of 15 secondary schools in the parish of Manchester. Over six academic years from 2007/8 to 2012/3, a total of 16 636 students – mostly aged 15–19 years – were screened with a single 5 mL EDTA blood tube which allowed haematology, electrophoresis and haemoglobin genotype confirmation (36). All students received laminated cards with personal information and their haemoglobin genotype and carriers of abnormal genes were offered counselling.

The stage was now set to determine whether knowledge of haemoglobin genotype in this 'informed cohort' influenced their choice of partner and reduced the frequency of births with sickle cell disease. Newborn screening was therefore established in the area where they were most likely to deliver their offspring, starting in Mandeville Regional Hospital in August 2008, extending to Percy Junor Hospital in Spalding, and a small private hospital (Hargreaves Memorial) in Mandeville in January 2009 and to May Pen and Black River Hospitals in January 2010. Together with the hospitals already screened in the corporate area, the total now increased to 23 000 or 58% of the estimated 40 000 all-island births in 2010.

By then, it was clear that newborn screening was so simple and cost-effective that the decision was made to offer this service to the western healthcare region, commencing with Savanna-la-Mar Hospital on December 4, 2013, Cornwall Regional Hospital on March 1, 2014, Noel Holmes Hospital (Lucea) on June 17, 2014, and Falmouth Hospital on July 1, 2014. Other smaller hospitals were gradually recruited including Royale Medical Centre in Savanna-la-Mar from January 2014, Lionel Town Hospital from May 2014 and Chapelton Hospital from April 2015, and nurse/midwives have collected 42 samples from home deliveries in the last year. These hospitals added a further 7000 births annually and the proportion of all-island deliveries screened, including those in the corporate area screened by the Sickle Cell Unit, increased to 30 000 or 81% of the estimated 37 000 births in 2013 (37). There remain 7000 births in Princess Margaret Hospital, Morant Bay, St Thomas, and the northeast healthcare region – St Ann's Bay, Port Maria, Annotto Bay, Port Antonio – and Linstead Hospitals which are due to be screened by the Sickle Cell Unit, Tropical Medicine Research Institute, The University of the West Indies before the end of 2015.

Logistical considerations

In the programme operated by the Sickle Cell Trust throughout the southern and western regions of Jamaica, dried blood samples are batched and conveyed to the central laboratory at the Southern Regional Health Authority in Mandeville. Samples from the southern region are delivered directly to this laboratory, whereas those from the western region are conveyed to the laboratory at Cornwall Regional Hospital and then to Mandeville at weekly intervals. On arrival, all demographic details from the Guthrie cards are entered in Excel files prior to analysis. Compliance with sample collection is monitored by audit of the delivery books at individual hospitals which allows exclusion of legitimately missed samples as in stillbirths or mothers delivering before arrival (BBA). These audits indicated that over the last year, mean collection rates have been maintained at 98% (range 95.4–99.7%), but they also provide a mechanism for identifying babies who were missed and so may be screened by heel-prick at the six-week clinic.

Confirmation

Sample confirmation covers two issues, firstly confirming the identity of the HbS band which is usually achieved by a laboratory method such as IEF or agar gel and secondly to confirm the identity of the child to whom the sample was attributed.

Most results are available within two weeks of sample collection and mothers are contacted as early as possible by phone through numbers recorded on the Guthrie cards; if this contact fails, additional numbers are sought from the Medical Records section of appropriate hospitals, and if contact still fails, the local police has been very helpful in visiting mothers to establish contact. This contact is initially made by the Educational Coordinator of the Sickle Cell Trust who has a background in genetic counselling and the laboratory suitable for providing answers to technical questions. All potentially clinically significant cases must be confirmed by blood tests when aged four to six weeks to allow their referral to local Sickle Cell Clinics by two months of age when pneumococcal prophylaxis should commence. There were 327 babies suspected to have clinically significant sickle cell syndromes (SS, SC, Sβ thalassaemia, S-variants), of which five died early, and the diagnosis was confirmed in the remaining 322 (98.5%) babies. In this confirmation process, parental blood tests can assist diagnosis (next section). Despite the high pressures in many maternity units, there has not been a single baby with disparate results suggesting error in sample collection or documentation, testimony to the diligence of Jamaican midwives and medical technologists.

Value of family studies

Parental blood tests may make useful contributions to the infants' genotype, sometimes avoiding complex and expensive confirmatory tests. Of the 167 babies with an SS phenotype, six were found on family studies to have the asymptomatic S-HPFH syndrome and in three, confirmation was not possible because of early death. In the remaining 158 with an SS phenotype, all were confirmed as SS disease by the subsequent evolution of haematology and clinical features, and the sickle cell gene was confirmed in 157 surviving mothers (152 AS, 1 $S\beta^{+}$ thalassaemia, 1 SC disease, 3 SS disease) and in 117 surviving fathers (112 AS, 2 SC disease, 2 SS disease, 1 S-HPFH). In the father with S-HPFH, the offspring could have been either SS or S-HPFH but the death from acute splenic sequestration at eight months confirmed SS disease. Of the 40 fathers not tested, 25 declined and 15 had inconsistent genotypes (AA or AC) casting doubt on paternity. The HPFH gene is more common in Jamaica than reports from the USA (38) and of the six cases with sickle cell-HPFH syndrome, the HPFH gene was found in two mothers and four fathers; this diagnosis would not have been apparent at this age without family studies. The diagnosis of sickle cell-β^{o} thalassaemia was based on the β^{o} thalassaemia gene, confirmed by DNA studies, in two mothers and three fathers.

Delivering the results

Contrary to programmes in the USA where the results of screening are conveyed to the family's physician, this would be inappropriate for most Jamaican families. In Jamaica, the mothers of babies with clinically significant genotypes are informed and requested to attend for confirmation within four to six weeks of birth to allow pneumococcal prophylaxis to commence by eight weeks. Other potentially significant genotypes have less urgency and there has been a policy to insert genotypes in the hospital records to clarify subsequent clinical events even if regular follow-up does not seem justified. Policy decisions are needed on returning the information for babies found to have HbS and HbC traits. These results could be entered in the child 'passport' but conveying this information to the family requires counselling to avoid misunderstanding the results. It may be questioned whether this information should be given to the families at this time since it will not have practical significance until the child grows up and forms their own social relationships. In the past, parental studies of babies with abnormal traits were proposed in order to identify parental relationships at risk for babies with clinically significant disease but serious social problems emerged when neither 'parent' was found to have the gene inherited by the child. Babies not showing abnormal haemoglobins must be considered as AA phenotypes since approximately 1% will carry beta thalassaemia or HPFH genes not readily detectable at birth. Further identification of rare haemoglobin variants is not entirely academic since one-third of variants generate clinical disease if inherited with HbS and so have potential clinical significance for the future. Collaboration with reference laboratories with appropriate DNA expertise may serve to identify these variants. If computer databases could be developed and maintained, this could store information on population genotypes to be made available when needed.

Reports

Quarterly reports are distributed to all collaborating hospitals, focusing on midwives, nurses and doctors involved in the maternity services. These serve the purpose of regular feedback on results, highlighting collection rates and problems such as maternal contamination, keeping staff motivated but also serve as an educational medium on recent technical advances and current issues.

Ethical issues

The extensive panel of metabolic and other disorders screened in the USA raises many ethical concerns which are beyond the scope of the present paper but currently, parents are informed of newborn blood collection but not routinely required to sign informed consent (39). The situation is simpler in Jamaica where screening is confined to haemoglobinopathies which offers clear benefits from early detection and follow-up. In the circumstances, the Jamaican Ministry of Health takes the position that the benefits of newborn screening far outweigh any potential disadvantages and written informed consent would not be required. Before delivery, patients are informed of newborn screening by the nurses and posters on the delivery wards provide further information. Dried blood spots are stored pending clarification of the diagnosis and then routinely incinerated.

RESULTS OF NEWBORN SCREENING IN JAMAICA

Genotype frequencies from newborn screening in south and west Jamaica are compared with the Jamaican Cohort Study in Table 1. The sickle cell trait occurred in 9.8%, the HbC trait in 3.8%, SS disease in 161, SC disease in 125 and sickle cell-beta thalassaemia in 36.

Table 1: Haemoglobin genotypes in two Jamaican studies of newborn screening

Genotype	Current study (4/8/08–31/3/15) Total (%) Observed	Adjusted*	Jamaican Cohort Study (25/6/73–28/12/81) Total (%)
AA (normal)	46 720 (85.62)	46 049 (84.39)	84 659 (84.38)
Sickle cell trait	5332 (9.77)	5332 (9.77)	10 049 (10.05)
HbC trait	2058 (3.77)	2058 (3.77)	3591 (3.59)
Beta$^+$ thalassaemia trait	–	447 (0.82)	556 (0.56)†
Beta^o thalassaemia trait	–	44 (0.08)	236 (0.24)†
HPFH trait	–	180 (0.33)	152 (0.15)†
SS disease	161 (0.30)	161 (0.30)	315 (0.32)
SC disease	125 (0.23)	125 (0.23)	201 (0.20)
S-beta$^+$ thalassaemia	31 (0.057)	31 (0.057)	33 (0.033)
S-beta^o thalassaemia	5 (0.009)	5 (0.009)	14 (0.014)
S-HPFH	6 (0.011)	6 (0.011)	9 (0.009)
CC disease	21 (0.038)	21 (0.038)	40 (0.040)
C-beta$^+$ thalassaemia	9 (0.016)	9 (0.016)	13 (0.013)†
C-beta^o thalassaemia	0	0	5 (0.005)†
C-HPFH	5 (0.009)	5 (0.009)	4 (0.004)†
Variant trait	88 (0.161)	88 (0.161)	111 (0.11)
S-Variant	5 (0.009)	5 (0.009)	8 (0.008)
C-Variant	0	0	4 (0.004)
Total	**54 566**	**54 566**	**100 000**

HPFH refers to the classical hereditary persistence of fetal haemoglobin
†genotypes estimated as described (28)
*genotypes partitioned according to frequencies observed in the school screening data (36)

Less common haemoglobin structural variants occurred in 112 (55 beta chain, 13 alpha chain, 19 gamma chain, 1 refused investigation, 24 awaiting identification) of which five were associated with HbS (1 SE, 1 SLepore Washington and 3 SO Arab). The 19 gamma chain variants will disappear and have been classified as AA phenotypes, which they will become. The uncommon beta chain structural variants and beta thalassaemia genes along with their mutations and their nomenclature as defined by the Human Genome Variation Society (HGVS) are shown in Table 2. The alpha chain variants are entirely benign and most of the beta chain structural variants are benign as traits or in combination with HbS. The variants HbO Arab, HbD Punjab, Hb Lepore Washington interact with HbS to cause significant disease and these accounted for 17/55 (31%) of the identified beta chain variants.

The genes for beta thalassaemia trait are not readily detectable at birth, appearing as AA phenotypes but partitioning according to the relative frequencies in the school screening data (36), suggest frequencies of 447 with beta$^+$ thalassaemia genes and 44 with beta^o thalassaemia trait. The spectrum of mutations (Table 2) was determined from babies inheriting sickle cell- and HbC-beta thalassaemia which revealed 10 different beta thalassaemia variants, their significance depending upon the degree of beta chain synthesis and hence HbA. When inherited with HbS, the mild promoter mutations, -29 A>G, -87 C>A, -88 C>T, and the PolyA T>C, which accounted for 34 of the 42 identified, produce 15–30% HbA in later childhood, sufficient to inhibit sickling and result in mild clinical syndromes. In association with HbS, the severe β^+ thalassaemia genes produce much lower levels of HbA and the β^o genes produce no HbA and so may be electrophoretically and clinically indistinguishable from SS disease. The genes for hereditary persistence of fetal haemoglobin (HPFH) are also difficult to detect at birth as heterozygotes and similar extrapolations were performed to indicate that 180 babies would be expected to inherit these genes.

The spectrum of structural and thalassaemic haemoglobin variants, derived from double heterozygotes with HbS or HbC, are consistent with the West African origin of most of the population, although HbD Punjab, HbE, IVSI-5 G>A, IVSI-5 G>C and the cd43 G>T mutations are more common in other ethnic groups, reflecting the heterogeneity of the Jamaican population. Gene frequencies obtained by gene counting (Table 3) and the derived genotype frequencies (Table 4) agree closely, as to be expected since one is derived from the other but this method serves to identify deviations from expected values for compound heterozygotes. Most agree closely, although observed cases of sickle cell-beta thalassaemia were more and of S-HPFH less than expected.

Table 2: Uncommon variant haemoglobins and beta thalassaemia genes found on newborn screening during the period 14/8/08–31/3/15

	Mutation	HGVS nomenclature	Clinical significance	Number
Alpha globin gene				
HbG Philadelphia	68(E17) Asn>Lys	HBA2:c.[207C>G (or HBA1) or 207C>A]	benign	13
Beta globin gene				
Structural haemoglobins				
HbJ Baltimore	beta 16(A13) Gly>Asp	HBB:c.50G>A	benign	1
HbE	beta 26(B8) Glu>Lys	HBB:c.79G>A	benign	7
Hb Osu Christiansborg	beta 52(D3) Asp>Asn	HBB:c.157G>A	benign	22
Hb Ocho Rios	beta 52(D3) Asp>Ala	HBB:c.158A>C	benign	1
Hb Korle Bu	beta 73(E17) Asp>Asn	HBB:c.220G>A	benign	3
Hb Caribbean	beta 91(F7) Leu>Arg	HBB:c.275T>G	benign	2
HbN Baltimore	beta 95(FG2) Lys>Glu	HBB:c.286A>G	benign	2
HbD Punjab	beta 121(GH4) Glu>Gln	HBB:c.364G>C	interacts with HbS	5
HbO Arab	beta 121(GH4) Glu>Lys	HBB:c.364G>A	interacts with HbS	11
Hb Lepore Washington	delta-beta hybrid	NG_000007.3:g.63632_71046del	severe β^o	1
Thalassaemia genes				
-88 C>T	beta nt -88 C>T	HBB:c.-138C>T	benign β^+	16
-87 C>A	beta nt -87 C>A	HBB:c.-137C>A	benign β^+	1
-29 A>G	beta nt -29 A>G	HBB:c.-79A>G	benign β^+	14
IVSI-5 G>A	beta nt 147 G>A	HBB:c.92+5G>A	severe β^+	2
IVSI-5 G>C	beta nt 147 G>C	HBB:c.92+5G>C	severe β^+	1
IVSII-5 G>C	beta nt 500 G>C	HBB:c.315+5G>C	severe β^+	1
IVSII-849 A>G	beta nt 1344 A>G	HBB:c.316-2A>G	severe β^o	2
PolyA T>C	beta nt 1584 T>C	HBB:c.*110T>C	benign β^+	3
106/107 +G	beta nt new stop codon	HBB:c.321_322insG	severe β^o	1
Cd43 G>T	beta 43(CD2) Glu>Stop	HBB:c.130G>T	severe β^o	1

Table 3: Gene frequencies derived from adjusted frequencies in Table 1

	Genes counted	Gene frequencies
A gene	100 247	0.918585
S gene	5826	0.053385
C gene	2239	0.020516
Beta thalassaemia	536	0.004912
HPFH	191	0.001750
Rare variants	93	0.000852
Total	**109 132**	**1.0000000**

Comparison with Jamaican Cohort Study

Comparison of the adjusted data in the current study with that obtained 35 years earlier in the screening to establish the Jamaican Cohort shows marginal differences in the frequency of the sickle cell trait and HbC trait but the only significant difference was the higher frequency of the haemoglobin variant trait in the current study (incidence rate ratio 1.39, 95% CI 1.04,1.84; $p = 0.02$). However, this difference may disappear since some of the variants not yet identified will be gamma chain variants and should therefore be classed with the AA phenotype. The differences in the beta thalassaemia trait may have reflected the dissimilar extrapolations for these frequencies but the ratio of beta$^+$ to beta^o genes at 2.3:1 in the first study was lower than the 10:1 in the latter. Furthermore, the molecular mutations differed, the -29 A>G accounting for 59% of Cohort beta$^+$ mutations (40) compared with 33% in the current study.

Table 4: Genotype frequencies derived from gene frequencies in Table 3

	Genotypes		
	Expected	Observed	%
AA	46 042.70	46 049	84.391
AS	5351.69	5332	9.772
AC	2056.67	2058	3.772
Beta thal trait	492.43	491	0.900
HPFH trait	175.43	180	0.330
SS	155.51	161	0.295
SC	119.53	125	0.229
Sbeta thal.	28.62	36	0.066
S-HPFH	10.20	6	0.011
CC	22.97	21	0.038
Cbeta thal.	11.00	9	0.016
C-HPFH	3.92	5	0.009
Variant trait	85.41	88	0.154
S-variant	4.96	5	0.009
C-variant	1.91	0	–
Others (HPFH/HPFH, *etc*)	3.05	0	–
Total	**54 566**	**54 566**	**99.992**

There were no differences between the other genotypes although the absence of C-beta^o thalassaemia in the current study raises the possibility that this accounted for some of the CC phenotypes, not confirmed by family study. Differences between these studies are more likely attributable to different populations with different founder effects rather than a true secular change.

Comparison with Guadeloupe

The other substantial body of newborn data from the Caribbean derives from the 27-year programme in Guadeloupe (21), where the frequencies of the sickle cell trait, HbC trait, SS disease, SC disease, sickle cell-beta thalassaemia, and haemoglobin variant traits were all significantly lower than in the Manchester study ($p < 0.001$). In Guadeloupe, the frequencies of SC and CC disease exceeded values predicted by the Hardy-Weinberg (HW) hypothesis, a difference, the authors believed, was most readily explained by underestimation of the HbC trait frequency. Among the less common haemoglobin variants, HbD Punjab dominated in Guadeloupe, whereas of the 55 beta chain haemoglobin variants identified in Jamaica, HbD Punjab accounted for 9% and Hb Osu Christiansborg for 40%. The frequency of the HPFH gene was similar in Jamaica and Guadeloupe and higher than reported elsewhere (38). Since the beta thalassaemia trait is not readily detectable at births, it must be extrapolated, and the gene frequency of 0.0028 derived from the HW hypothesis in Guadeloupe was nearly half the 0.0049 observed in Jamaica extrapolated from the frequency of beta thalassaemia syndromes. The spectrum of molecular mutations varied, the -29 A>G dominating in Guadeloupe, and the -88 C>T in Jamaica.

CONCLUSIONS

Newborn screening for sickle cell disease using dried blood spots is a simple and relatively cheap technology readily implemented in the Caribbean. All cases with potentially clinically significant genotypes should be confirmed by a heel-prick on the infant and parental tests may assist in the diagnosis. Once confirmed, all cases should be referred to sickle cell clinics for appropriate clinical care.

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