Newborn screening for sickle cell disease in Jamaica: logistics and experience with umbilical cord samples

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Received: 7 July 2016 / Accepted: 9 October 2016 / Published online: 29 October 2016
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Abstract The study aims to describe the logistics and results of a programme for newborn screening for sickle cell disease based on samples from the umbilical cord. Samples were dried on Guthrie cards and analysed by high pressure liquid chromatography. All suspected clinically significant abnormal genotypes were confirmed by age 4–6 weeks with family studies and then recruited to local sickle cell clinics. The programme has screened 66,833 samples with the sickle cell trait in 9.8 % and the HbC trait in 3.8 %. Sickle cell syndromes occurred in 407 babies (204 SS, 148 SC, 35 Sbeta⁺ thalassaemia, 6 Sbeta^o thalassaemia, 6 sickle cell-variants, 8 sickle cell-hereditary persistence of fetal haemoglobin) and HbC syndromes in 42 (22 CC, 14 Cbeta⁺ thalassaemia, 1 Cbeta^o thalassaemia, 5 HbC- hereditary persistence of fetal haemoglobin). Focusing on the year 2015, screening was performed in 15,408, compliance with sample collection was 98.1 %, and maternal contamination occurred in 335 (2.6 %) but in only 0.05 % did diagnostic confusion require patient recall and further tests. This model of newborn screening for sickle cell disease is accurate, robust and economic. It is hoped that it may be helpful for other societies with high prevalence of abnormal haemoglobins and limited resources, who are planning to embark on newborn screening for sickle cell disease.

Keywords Newborn screening · Sickle cell disease · Jamaica · Umbilical cord samples

Introduction

The benefits of newborn screening for sickle cell disease are indisputable, and early detection has been widely implemented. Such screening is usually integrated into preexisting programmes detecting inborn errors of metabolism of which two of the more common, phenylketonuria and galactosaemia, require a milk feed before sample collection. This sample is therefore taken by heel prick 2–5 days after birth and the same dried blood spots used for detection of other conditions including sickle cell disease. Where newborn screening for sickle cell disease is not part of established programmes screening for other conditions, sample collection from the umbilical cord has the advantages of ease and simplicity but potential disadvantages of contamination by maternal blood. Jamaican programmes for newborn screening (Serjeant et al. 1974; Serjeant et al. 1986; King et al. 2007) have been based on umbilical cord samples and the experience may be useful for the development of similar programmes elsewhere.

Newborn screening for sickle cell disease is vital to allow implementation of interventions known to reduce early morbidity and mortality. Jamaica was the first country in the world to have extensive newborn screening with the programme based at Victoria Jubilee Hospital (the main Government Maternity Hospital in Kingston) which screened 100,000 consecutive births between June 1973 and December 1981. In 1987, a conference at the National Institutes of Health in Bethesda concluded that ‘the benefits of screening are so compelling that universal screening should be provided’ (Consensus Conference 1987). Screening for much rarer inborn errors of metabolism was already underway in the USA, and screening

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for sickle cell disease was gradually added becoming universal in 2006 in the USA and in 2005 in England. In Jamaica, the lack of preexisting programmes screening for metabolic disorders allowed screening for sickle cell disease based on cord blood samples with some novel and cost effective features which may assist the development of similar programmes elsewhere. The techniques and logistics of the Jamaican screening programme are therefore analysed and presented in some detail.

History of newborn screening for sickle cell disease in Jamaica

Newborn screening was instituted at Victoria Jubilee Hospital in Kingston in 1973 and in the next 8.5 years, 100,000 consecutive non-operative deliveries were screened in order to establish the Jamaican Cohort Study of sickle cell disease. Screening was based on liquid blood samples taken in EDTA as the umbilical cord was cut, and the short duration of admission mandated that samples were collected daily including weekends and bank holidays. Daily auditing of the ward delivery records identified missed samples which were then collected by heel prick. The samples were analysed daily by haemoglobin electrophoresis on cellulose acetate, allowing mothers to be informed of the presumptive diagnosis before hospital discharge. At weekly intervals, all electrophoretic abnormalities were subjected to agar gel electrophoresis at pH 6.2 (Serjeant et al. 1974). The distribution of genotypes for the 100,000 samples (Serjeant et al. 1986) revealed that the sickle cell trait occurred in 10.0 % and the HbC trait in 3.5 % and the programme recruited 550 cases of sickle cell disease (311 homozygous sickle cell (SS) disease, 201 sickle cell-haemoglobin C (SC) disease, 34 with sickle cell-beta⁺ thalassaemia, and 13 with sickle cell-beta^o thalassaemia) and a group of 250 controls with an AA phenotype.

Screening at Victoria Jubilee Hospital ceased in December 1981 but was re-established with government support on 1 November 1995 and later extended to other public hospitals in the corporate area (University Hospital from 23 October 1997 and to Spanish Town Hospital from April 15, 1998). Dried blood samples were now collected on Guthrie cards, eluted and analysed as before by cellulose acetate and agar gel electrophoresis. Total deliveries at these three institutions were 15–16,000 annually or approximately one third of all island deliveries and on the cessation of the Medical Research Council Laboratories in October 1999, this programme continues under the Sickle Cell Unit of the Tropical Medicine Research Institute of the University of the West Indies.

The Manchester Project in central Jamaica commenced in 2008 with the objective of determining whether knowledge of haemoglobin genotype in secondary school students would influence their choice of partner and reduce the births affected by sickle cell disease. Coincident with the school screening

phase (Mason et al. 2015), newborn screening was instituted at Mandeville Regional Hospital serving Manchester parish from August 2008 and, over the next 5 years, progressively extended to 12 hospitals throughout the south and west of Jamaica. Dried blood samples were collected on Guthrie cards and relayed to a central laboratory in Mandeville where initially samples were analysed by agar gel electrophoresis but after November 2008 by high-pressure liquid chromatography (Bio-Rad Variant, Bio-Rad NBS) (Mason et al. 2016). This programme now screens over 15,000 babies or 42 % of the estimated 36,000 annual deliveries in Jamaica.

The Sickle Cell Unit at the University of the West Indies continues to screen deliveries in the Corporate area, adding hospitals in the northeast of the island in October 2015 and in the southeast in November 2015 so 42 years after it began to screen, Jamaica has finally achieved near universal screening for sickle cell disease.

Production of filter paper (Guthrie) cards

It proved feasible and economical to produce Guthrie cards locally, the printed clerical section measuring 105 × 50 mm being adhered to a filter paper section measuring 30 × 50 mm of which 22 × 50 mm was exposed and available for the blood sample. The filter paper section was made from chromatography #3MM paper and a box of 100 sheets measuring 45 × 57 cm produced 14,000 cards. The final cost including filter paper, printing the clerical section and adhering together was US\$0.06 per card.

Maternal contamination

The amount of HbA in babies with an AA phenotype at birth is influenced by gestational age and the possibility of maternal contamination in cord blood samples. Information on gestational age is rarely available and a method for the quantitation of the HbA from maternal contamination has been explored. Defining the amount of HbA by the ratio of HbA/HbF1 + HbF ≥ 0.50 (where HbF1 represented acetyl HbF with a retention time of 0.29–0.35), correlated well with the visual assessment of contamination. Using this criteria, maternal contamination was estimated to occur in 2.6 % samples (range 1.7–3.7 % in different hospitals) (Table 1). The importance of maternal contamination is in its potential for diagnostic confusion but this does not occur in 85 % of samples in Jamaica where both mother and baby would be expected to have an AA phenotype. Confusion may occur where mother or baby have abnormal genes and 8 were recalled for diagnostic clarification because of contamination by AS or AC maternal genotypes. A further 9 were recalled for elucidation of variant bands but these were not attributable to contamination.

Table 1 Newborn screening by umbilical cord blood: data for 2015

Hospital	Deliveries				Collection	Maternal contamination	
	Total	SB/ BBA	Eligible	Missed	Compliance	AF bands*	Recalled
Southern region							
Hospital 1	3888	65	3823	91 (2.4 %)	3732 (97.6 %)	80/3170 (2.5 %)	3
Hospital 2	2765	34	2731	83 (3.0 %)	2648 (97.0 %)	61/2207 (2.8 %)	1
Hospital 3	1186	27	1159	21 (1.8 %)	1138 (98.2 %)	28/957 (2.9 %)	3
Hospital 4	323	4	319	5 (1.6 %)	314 (98.4 %)	10/272 (3.7 %)	0
Western Region							
Hospital 5	4166	75	4091	54 (1.3 %)	4037 (98.7 %)	91/3438 (2.6 %)	0
Hospital 6	2462	18	2444	14 (0.6 %)	2430 (99.4 %)	35/2062 (1.7 %)	1
Hospital 7	726	13	713	15 (2.1 %)	698 (97.9 %)	21/586 (3.6 %)	0
Hospital 8	423	4	419	8 (1.9 %)	411 (98.1 %)	9/351 (2.6 %)	0
Total	15,939	240	15,699	291 (1.9 %)	15,408 (98.1 %)	335/13,043 (2.6 %)	8 (0.05 %)

SB/BBA stillbirths/born before arrival, eligible deliveries computed after deduction of SB/BBA

Hospitals 1 and 6 include data for small private hospitals; hospital 2 includes data for two small Government Hospitals

Maternal contamination, AF bands* analysed in samples with AA haemoglobin phenotype with HbA/HbF + F1 ratio ≥ 0.50

Compliance with sample collection

The inevitable inaccuracies in the manually maintained delivery books on the maternity wards meant that accurate compliance with sample collection could only be derived by regular audits of delivery books conducted by phone or by periodic visits to the larger hospitals. Deliveries were considered eligible for sample collection after excluding stillbirths or babies born before arrival (BBA) and eligible samples not collected were defined as ‘missed’. Inevitably ‘missed’ samples were common at the beginning of recruitment of new hospitals and in the three larger hospitals monitored over 5 years, missed samples fell from 8.3 % in the first year to 1.7 % in the 5th year probably the result of continued feedback and encouragement through regular reports. In the last year, compliance was maintained in excess of 98 % and could be improved further by heel prick sampling of babies missed at birth. Causes of missed samples were obstetric (postpartum haemorrhage, Caesarean sections) or paediatric emergencies (admission to special care nursery, congenital abnormalities), and failure to maintain supplies of Guthrie cards. Caesarean sections accounted for 71/291 (24 %) of missed samples.

Focusing on 2015, there were 98 samples from deliveries which did not appear in the maternity ward delivery books, representing 0.61 % of all deliveries. Some may have represented inadequately documented domiciliary deliveries but this discrepancy could add approximately 220 babies to the current annual estimate of 36,000 deliveries in the island.

Confirmation of haemoglobin genotypes

Protocols required confirmation of all potentially clinically significant genotypes and haemoglobin variants along with parental studies, where possible, within 4–6 weeks of birth in order to allow cases of sickle cell disease to commence pneumococcal prophylaxis by 2 months of age. Parental studies involved venepuncture samples for routine haematology and haemoglobin electrophoresis with confirmation of bands in the position of HbS by the sickle test and of HbC by agar gel. Parents with an AA phenotype and an MCH ≤ 26 pg and RDW < 18 were considered candidates for beta thalassaemia and HbA₂ was measured which if ≥ 3.5 % led to DNA sequencing. For parents with a prominent band in the position of fetal haemoglobin (HbF) this was quantitated and samples with values > 15 % had DNA sequencing to identify HPFH genes. Family studies were particularly helpful in detecting conditions such as sickle cell-hereditary persistence of fetal haemoglobin (S-HPFH), a condition mimicking SS disease electrophoretically, but haematologically and clinically near normal. The HPFH gene occurred in 55/16,636 (0.33 %) students in the Manchester Project (Mason et al. 2015) which is more common than previous estimates among African-Americans. Of 393 babies with the four major genotypes of sickle cell disease (204 SS, 148 SC, 35 S β^+ thalassaemia and 6 S β^0 thalassaemia), genotype confirmation was sought in 388 babies (five babies died before 7 days) by blood tests in 385 (99.2 %) mothers (1 died, 2 declined) and in 279

(71.9 %) fathers. Greater effort was put into tracing fathers of SS babies who were tested in 165/204 (81 %) compared with fathers of SC babies who were obtained in 78/148 (53 %). However, impaternalty remains a concern and probably accounted for the AA genotype in 18 (11 %) ‘fathers’ of SS offspring and in 6 (8 %) of SC offspring. In the 41 subjects with sickle cell-beta thalassaemia, the beta thalassaemia gene was demonstrated in 40 parents (20 mothers, 20 fathers) and in one case with an ‘AA father’ by detection of the –88 C > T mutation in the baby. Eight subjects were shown to have sickle cell-hereditary persistence of fetal haemoglobin (S-HPFH) by the HPFH gene in a parent (3 mothers, 5 fathers).

Techniques of genotype analysis

Dried blood samples were cut out from the Guthrie cards, eluted in water in glass test tubes and an aliquot of haemolysate placed in the well plates of the Bio-Rad NBS high-pressure liquid chromatography system. Haemoglobin peaks were identified by characteristic retention times for HbF, HbA, HbS, HbC, and peaks with other retention times were assumed to be rare variant haemoglobins. When detected by family study, the genes for beta thalassaemia and HPFH were sequenced for confirmation. Rare variants were also sequenced which allowed separation into variants of the alpha or beta chain. Variants which occurred in neither parent or disappeared with age were assumed to be gamma chain variants but have not yet been identified.

Distribution of haemoglobin genotypes

The validity of haemoglobin genotype determination at birth (Table 2) was tested by comparison with published data from school screening in the same population (Mason et al. 2015) which shows close agreement in the frequency of the sickle cell trait (9.82 %) and of the HbC trait (3.76 %) giving gene frequencies of 0.05365 and 0.02041 for S and C respectively. These gene frequencies predict 192 SS, 146 SC and 28 with CC disease suggesting a slight excess of the SS phenotype, some of which may resolve into other genotypes on follow-up. The prevalence for all genotypes from newborn screening matched closely those in the school population except for SS disease where the number was 44 % below that expected from gene frequency (Mason et al. 2015), possible causes being early death, default from school, or reluctance for a further test in those already known to have SS disease. The genes for β -thalassaemia and hereditary persistence of fetal haemoglobin are not readily detectable at birth and have been interpolated from the trait frequencies observed in the school population. Evaluation of the prevalence of the rare haemoglobin variants is sometimes complicated by lack of knowledge of the affected

Table 2 Distribution of beta globin genotypes at birth by 31 December 2015 and in school screening in the same population

Genotype/phenotype	Number	%	School	%
AA ^a	56,392	84.377	14,138	84.984
AS	6559	9.814	1601	9.624
AC	2516	3.765	582	3.500
β^+ thalassaemia	(551)	(0.824)	137	0.824
β^0 thalassaemia	(52)	(0.078)	13	0.078
HPFH trait	(221)	(0.331)	55	0.331
SS	204	0.305	31	0.186
SC	148	0.221	37	0.224
S β^+ thalassaemia	35	0.052	9	0.054
S β^0 thalassaemia	6	0.009	1	0.006
S-HPFH	8	0.012	3	0.018
S-variant	6	0.009	1	0.006
CC	22	0.033	9	0.054
C β^+ thalassaemia	14	0.021	0	–
C β^0 thalassaemia	1	0.001	1	0.006
C-HPFH	5	0.007	0	–
HPFH/HPFH	1	0.001	0	–
Variant traits ^b	92	0.138	18	0.108
Total screened	66,833	99.998	16,636	100.003
Unknown	13			
Stillbirths/born before arrival	1164			
Missed samples	2041			
Grand total	70,051			

^a Includes 15 alpha chain variants and 23 presumed gamma chain variants

^b Ninety-two variant traits include 57 identified beta chain variants, 4 unknown (2 died, 2 declined investigation), and 31 await identification which may indicate alpha, beta, or gamma chains

‡Babies unavailable for confirmation (early death, unable to contact, refused further tests)

HPFH hereditary persistence of fetal haemoglobin. The genes for beta thalassaemia and HPFH are not readily detected at birth, and the relative prevalence, shown in *brackets*, has been extrapolated from school students in the same population

globin chain. In 23 subjects with variant bands, the lack of haemoglobin variants in all mothers and in 11 fathers, the failure to detect variants with probes for the alpha and beta globin variants, and their disappearance with age was consistent with gamma chain variants and these were classified as the relevant beta chain (22AA, 1AS). The 15 alpha chain variants, all HbG Philadelphia, were associated with a normal beta globin genotype. There remained 92 babies with haemoglobin variants, of whom 4 died or further investigation was declined, 57 had beta chain variants and 31 await analysis, some of which will be shown to be variants of the alpha or gamma chain. Of the 13 subjects without diagnosis, 5 had died before recall, 6 could not be contacted, and 2 refused investigation.

Targeting newborn screening by maternal genotype

Assuming that the primary purpose of screening is the detection of clinically significant genotypes, it could be argued that in Jamaica where 85 % of the population has a normal AA phenotype, targeting the offspring of the 15 % of mothers carrying abnormal genes would save resources. This would require definitive identification of the maternal haemoglobin genotype and access to this information at the time of delivery. The genotype for major abnormal haemoglobin such as HbS and HbC, if not previously known, could be derived during antenatal care but the beta thalassaemia trait suspected on microcytic red cell indices is a greater challenge with the iron deficiency common in pregnancy. Attitudes will be determined by the prevalence and pattern of beta thalassaemia genes in a community. In Jamaica, these occur in less than 1 % of the population, and since 33/41 (80 %) are mild beta⁺ mutations (−90 C>T, −88 C>T, −87 C>A, −29 A>G and PolyA T>C) it could be argued that few seriously affected cases would be missed. On balance and bearing in mind the low cost of Guthrie cards compared to HPLC reagents, the most realistic and economic approach would be collection of samples from all births and retrieval of the mother's genotype prior to HPLC analysis. This approach could save US\$9000 or 22 % of the US\$42,000 annual cost of the current newborn screening in the south and west of Jamaica.

Disclosure of results of newborn screening

Clearly genotypes of potential clinical significance must be notified to the family early to allow confirmation and recruitment to special clinics, pneumococcal prophylaxis and education for the family. The issues are less clear for the disclosure of other results to the family and policies vary elsewhere according to resources. In Jamaica, AS and AC genotypes are not routinely disclosed to the families partly because of the lack of education and counselling resources which would be needed to prevent misunderstanding and misinterpretation of the result but also because this information may not be of practical value until the child reaches reproductive age. This problem is further compounded by the failure to detect the genes for beta thalassaemia and HPFH in newborn samples and although these may be uncommon, this source of inaccuracy may have serious implications for later genetic counselling. Rare haemoglobin variants may present similar problems and it could be argued that their identification is unrealistic technically or financially especially as the majority have no clinical significance but in Jamaica, one-third of beta globin variants, HbD Punjab, HbO Arab and Hb Lepore, may interact with HbS to produce clinically significant disease.

Comment

The newborn screening programme for sickle cell disease initiated by the Sickle Cell Trust (Jamaica) has operated effectively, efficiently and economically for the last 8 years. It is based entirely on samples collected from the umbilical cord and maternal contamination causing diagnostic confusion occurred in only 0.05 %. Compliance with sample collection has been excellent at over 98 % and could be improved further with the introduction of heel pricks on babies who were missed. The programme has detected 406 babies with clinically significant abnormal genotypes excluding 13 cases with S-HPFH and C-HPFH which have little, if any, clinical significance. It could be argued that distinction of genotypes within the SS phenotype is not essential for clinical care since most would receive pneumococcal prophylaxis and other supportive measures but such distinction is useful for research purposes. The programme has also detected 130 rare haemoglobin variants, 15 affecting alpha chains (all HbG Philadelphia), 57 of the beta chain (10 different variants), 23 believed to affect the gamma chains, 30 are awaiting analysis, and 5 died or declined further investigation. Identification of variants adds expense and has limited clinical significance as all are asymptomatic as traits although HbO Arab, HbD Punjab, and Hb Lepore interact with HbS to produce significant disease and these three variants accounted for 17/57 (30 %). The decision on whether to pursue and identify such variants would be determined by their frequency and distribution in different societies and on the resources for investigation, but freezing such samples for long-term storage would allow the option of later identification.

Conventional wisdom of screening and confirmation by two separate and expensive laboratory methods (Ryan et al. 2010) has not proved necessary or feasible in Jamaica where confirmation is achieved by parental tests or follow-up haematology in babies where biological fathers were not available.

Acknowledgments This work was supported by the National Health Fund of Jamaica, the Chase Fund of Jamaica, and the Alcoa Foundation. The authors wish to acknowledge the contributions of Professor Douglas Higgs and Dr. Chris Fisher of the Institute of Molecular Medicine, Oxford, for the identification of haemoglobin variants and of Professor Andreas Kulozik and Ms. Margit Happich of the Department of Pediatric Oncology, Hematology and Immunology, University of Heidelberg, Germany, for the identification of the beta thalassaemia molecular mutations.

Compliance with ethical standards The Jamaican Ministry of Health has taken the position that the benefits of newborn screening for sickle cell disease so far outweigh any risks that screening would be offered as a free service without the need for signed consent.

Conflict of interest Graham Serjeant, Beryl Serjeant, Karlene Mason, Ruth-Ann Gardner, Lansford Warren, Felicea Gibson, and Michael Coombs declare that they have no conflict of interest.

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