Screening for the beta-thalassaemia trait: hazards among populations of West African Ancestry

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Abstract The aim of this study was to examine the accuracy and characteristics of detecting the beta-thalassaemia trait in populations of West African ancestry. School children, aged 16–19 years, in Manchester Parish, Jamaica were screened to detect the genes which could give rise to offspring with sickle cell disease. Haematological indices and HbA₂ levels in subjects with an MCH $\leq$ 26 pg and an RDW $<$ 18.0 with DNA analysis in those with indices consistent with the beta thalassaemia trait were measured. The performance of published discriminant indices in distinguishing iron deficiency and beta-thalassaemia trait in this population was assessed. Of 10,148 subjects, 1,739 (17.1%) had an AA haemoglobin phenotype and red cell indices consistent with beta-thalassaemia (MCH values $\leq$ 26 pg, RDW $<$ 18.0) requiring estimations of HbA₂ levels. HbA₂ levels were $\geq$ 3.5% in 112 and beta-thalassaemia mutations were identified in 77 of these including the –88 C>T mutation in 35 (45%), –29 A>G in 19 (25%), –90 C>T in 7 (9%), the IVS II-849 A>G in 5 (6%) with smaller contributions from five other

mutations. Discriminant indices performed poorly in the differentiation of iron deficiency and the beta-thalassaemia trait. Detection of the beta-thalassaemia trait is relatively insensitive in populations of West African ancestry partly because of the mild defects characterising beta-thalassaemia in this population and also the high prevalence of deletional alpha thalassaemia. More sensitive indicators are required for beta-thalassaemia detection to inform such populations at risk of offspring with sickle cell disease.

Keywords Population screening · Beta-thalassaemia trait · Discriminant indices · West African ancestry

Introduction

Most clinically significant haemoglobinopathies result from the inheritance of abnormal haemoglobin genes from both parents and can therefore potentially be avoided if one parent is normal. This is the basis for prevention of affected births with beta-thalassaemia achieved by population screening in Sardinia (Cao et al. 1981) and Cyprus (Angastiniotis and Hadjiminis 1981; Angastiniotis et al. 1988). With the huge public health load of births with homozygous sickle cell (SS) disease in sub-Saharan Africa, conservatively estimated to exceed 250,000 births annually, prevention will be vital if the limited available resources are to provide adequate clinical care for patients. Population screening programmes are therefore designed to detect the genes which give rise to sickle cell disease in the hope of empowering carriers of abnormal genes to select partners with a normal haemoglobin (AA) genotype and avoid the risk of babies with sickle cell disease. Voluntary premarital screening in Bahrain has successfully reduced the frequency of births affected by sickle cell disease (Al Arrayed

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2005) and such screening has been mandatory in both Bahrain and Saudi Arabia since Royal Decrees in 2004. These predominantly Islamic societies are characterised by arranged marriages and following premarital screening of both prospective partners, a report that “this marriage is not recommended” infers that both carry abnormal haemoglobin genes and allows the families to make alternative arrangements. In Bahrain, this programme reduced the prevalence of births with SS disease from 2% to 0.9% during the voluntary trial period. However, it is unknown whether genotype identification and counselling in other societies would have a similar effect in reducing affected births, an important question with the frequency of sickle cell disease in high prevalence areas.

The Manchester Project in central Jamaica addresses this question by seeking to identify carriers of abnormal haemoglobin genes among senior school students rendering them at risk of having babies with sickle cell disease and counselling them so that they may make informed reproductive decisions. In Jamaica, the most common such genes are HbS, HbC and beta-thalassaemia. The first two are readily identified by alkali haemoglobin electrophoresis and confirmatory tests but the beta-thalassaemia genes have presented some difficulty, partly due to the mild haematological changes induced by the relatively mild beta-thalassaemia mutations occurring in populations of predominantly West African ancestry. Experience in their detection in the Manchester Project is therefore reviewed to assist others in the design of population screening programmes in these populations.

Material and methods

Manchester Parish is in south central Jamaica and ranges in altitude from sea level to over 3,000 ft with a total population of approximately 190,000 in the 2001 census. The target population were the fifth and sixth forms (grades 11–13), mostly aged 16–19 years, attending 15 secondary schools in the parish. Screening programmes commenced in January 2008 and this report covers data collected up to February 2011. After sensitisation procedures which included discussions with the school staff, presentations to Parent–Teachers Associations, illustrated lectures to the students on sickle cell disease and its genetics, and letters to the parents allowing them to opt-out of the screening if desired, screening was offered at arranged sites in each school. Demographic data forms were completed by the students and a team of three experienced phlebotomists with support staff collected 5 ml EDTA samples by venepuncture. Depending on the school, up to 250 samples could be collected within a 2 to 2.5-h interval, stored in cool boxes and returned to the central laboratory for sorting

against the pre-recorded class lists and allocation of sequential unique identification numbers.

All samples had haematological indices determined in an electronic cell counter (Abbott 1700 Cell-Dyn) and haemoglobin electrophoresis performed on mylar-backed cellulose acetate (Helena Laboratories). Bands in the position of HbS were confirmed by the slide sickle test and HbC by electrophoresis on acid agar gel at pH 6.2. All samples behaving atypically were studied by agar gel and quantification by high-pressure liquid chromatography (HPLC, Bio-Rad Variant), frozen and referred to specialist laboratories for final identification. The beta-thalassaemia trait was suspected in samples with an AA phenotype and mean cell haemoglobin (MCH) levels ≤ 26 pg and such samples had quantifications of HbA₂ performed by microcolumns (Helena Laboratories) before January 2009 and by HPLC afterwards. Samples with HbA₂ levels $\geq 3.5\%$ had excess plasma removed, were frozen and shipped in dry ice to the collaborating laboratory (Department of Pediatric Oncology, Hematology and Immunology Children's Hospital Im Neuenheimer Feld 430 D-69120 Heidelberg, Germany) where DNA sequencing was carried out to identify beta-thalassaemia mutations.

The ability of published discriminant functions to distinguish beta-thalassaemia trait from iron deficiency based on variables including mean cell volume (MCV), MCH, total haemoglobin (Hb), red cell count (RBC) and RDW was tested in a subgroup of these subjects. For this analysis, the iron-deficient group was confined to 80 subjects with an RDW (CV) ≥ 18.0 , low haemoglobin levels, characteristic microcytic and hypochromic indices, and in the majority of cases, correction of these abnormalities by oral iron supplementation and the beta-thalassaemia trait group was confined to 77 subjects with demonstrated molecular mutations.

Finally, all subjects received laminated cards bearing personal information and haemoglobin genotype. Individuals carrying abnormal genes received counseling and explanatory leaflets to take home to their families.

Results

The total population for which haematological indices were available was 10,148 (2,117 in academic year 2007/8, 2,626 in 2008/9, 2,621 in 2009/10 and 2,784 in 2010/11). Of these, 8,805 (86.8%) had an AA phenotype, manifesting HbA, HbF and HbA₂ on electrophoresis, but 37 of these had findings consistent with the gene for classic hereditary persistence of HbF leaving 8,768 eligible for the diagnosis of the beta-thalassaemia trait. Of these, 2020 (19.9%) had MCH values ≤ 26 pg and to exclude possible cases of iron deficiency, this group was further restricted to those with a

RDW <18.0 leaving 1,739 (17.1%) for further study. Of these, HbA₂ estimations in 1,694 showed 112 with values $\geq 3.5\%$, most of whom were referred for further identification. The distribution of results (Table 1) indicate that beta-thalassaemia mutations were not observed with HbA₂ levels below 3.5% but that the yield increased progressively above 4.0%. The molecular mutations along with HbA₂ and HbF levels (Table 2) shows that the -88 C>T mutation accounted for 35 (45%), the -29 A>G for 19 (25%), -90 C>T for 7 (9%), the IVS II-849 A>G for 5 (6%) with smaller contributions from five other mutations.

A confounding factor in the specificity of a low MCH as an indicator for beta-thalassaemia was the unexpectedly high prevalence of indices suggestive of iron deficiency among students aged 16–19 years. An RDW ≥ 18.0 , used as a surrogate marker of iron deficiency, occurred in 287 (2.8%) of the total group and occurred in 243 females (Hb range 4.5–13.1 g/dl) and 44 males (Hb range 6.3–13.8). Students with haemoglobin levels <10.0 g/dl received nutritional advice and iron supplementation which usually elicited a dramatic increase in haemoglobin level on repeat testing confirming a role for iron deficiency.

Indices proposed for discriminating the beta-thalassaemia trait from iron deficiency did not work well in this predominantly West African population (Table 3). The indices proposed by Ricerca et al. (1987), Shine and Lal (1977) and Green and King (1989) worked reasonably well for detecting beta-thalassaemia traits and most except those proposed by Shine and Lal (1977) and Srivastava and Bevington (1973) were sensitive for detecting iron deficiency. Overall, the most

accurate indices appeared to be those proposed by Green and King (1989) and Ricerca et al. (1987).

Discussion

The beta-thalassaemia trait has been estimated to occur in 1.5% of Jamaican newborns in Kingston, approximately 1.0% with beta⁺ thalassaemia and 0.5% with beta^o thalassaemia (Serjeant et al. 1986). These figures were projected from a relatively small sample of subjects with sickle cell-beta⁺ and sickle cell-beta^o thalassaemia and the prevalence had wide confidence intervals. The present study identified a frequency of 0.76% or approximately half that expected which could reflect a more accurate prevalence or a true difference between populations in Kingston and Manchester Parish. Furthermore, mutations characterised by mild beta⁺ thalassaemia (-88 C>T, -29 A>G, -90 C>T, PolyA T>C) accounted for 64 (83%) of mutations compared with 13 (17%) with the beta^o or severe beta⁺ mutations (IVS II-849 A>G, IVS I-108 T>C, IVS I-110 G>A, IVS I-5 G>A, IVS II-781 C>G) indicating a ratio in favour of beta⁺ genes of 5:1 compared to previous estimates of 2:1. Overall, it seems more likely that this is a truer prevalence of the beta-thalassaemia genes in Jamaica.

The spectrum of beta-thalassaemia mutations reflect the diversity of the Jamaica population, which, although predominantly of West African origin, contains contributions from many other racial groups including countries bordering the Mediterranean, India and China. The detection of the beta-thalassaemia trait remains a challenge especially in populations of West African origin characterised by mild beta⁺ thalassaemia genes. The initial screening by an MCH ≤ 26 pg was non-specific since 20% of those with an AA phenotype had low values. This low specificity partly results from iron deficiency and a high prevalence of alpha thalassaemia in this population. Iron deficiency, based on an MCH ≤ 26 pg and an RDW ≥ 18.0 , accounted for 2.8% of the overall group and 14.2% of those with MCH values ≤ 26 pg. Heterozygotes and homozygotes for alpha thalassaemia account for 31% and 2%, respectively of those with an AA phenotype and for 34% and 3% of those with SS disease in Kingston newborns (Serjeant et al. 1986). The prevalence of alpha thalassaemia is not available in this Manchester population but inferring from the data collected in Kingston, it is likely that either iron deficiency or alpha thalassaemia accounts for approximately half the subjects with low MCH values. The lack of specificity of the low MCH requires many estimates of HbA₂ at considerable cost and even those with HbA₂ levels $\geq 3.5\%$ showed beta-thalassaemia mutations in only 77 (73%) of 106 samples referred for structural analyses.

Table 1 Distribution of HbA₂ levels and beta-thalassaemia genes in subjects with HbA₂ levels $\geq 3.0\%$

HbA ₂ level (%)	Total subjects	Not submitted for molecular studies	No mutation found	Beta-thalassaemia gene confirmed
3.0–3.4	305	297	8	0
3.5–3.9	32	10	18	4
4.0–4.4	3	0	2	1
4.5–4.9	8	0	0	8
5.0–5.4	26	0	0	26
5.5–5.9	27	1 ^a	1	25
6.0–6.4	11	2 ^a	0	9
6.5–6.9	5	1 ^a	0	4
Total	417	311	29	77

^a These represent samples which were mistakenly omitted from molecular analysis

Table 2 Molecular mutations with HbA₂ and HbF distributions

Mutation	Number	HbA ₂ Mean (median), SD, range	HbF Mean (median), SD, range
–88 C>T	35	5.57 (5.5), 0.58, 3.5–6.8	4.88 (5.2), 2.60, 0.9–9.1
–29 A>G	19	5.38 (5.4), 0.52, 4.6–6.5	1.32 (1.5), 2.44, 0.8–8.2
–90 C>T	7	5.43 (5.5), 0.45, 4.7–6.0	2.61 (1.4), 2.56, 0.6–7.6
IVS II-849 A>G	5	5.56 (5.6), 0.21, 5.3–5.8	1.33 (1.3), 0.74, 0.6–2.1
PolyA T>C	3	4.67 (4.9), 0.59, 4.0–5.1	0.2 (–), –, 0.2
IVS I-108 T>C	2	4.10 (4.1), 0.85, 3.5–4.7	–
IVS I-110 G>A	2	5.60 (5.6), 0.57, 5.2–6.0	–
IVS I-5 G>A	2	5.10 (5.1), 0, 5.1–5.1	–
IVS II-781 C>G	2	3.55 (3.6), 0.07, 3.5–3.6	–
Total	77		

Quantification of HbA₂ is the most accurate indicator of the beta-thalassaemia trait (Table 1), molecular mutations increasing with HbA₂ levels and occurring in all but three subjects with HbA₂≥4.0% and in these three, HbA₂ levels were determined by the intrinsically less accurate micro-column method. Since HbA₂ quantification emerges as the most specific indicator of beta-thalassaemia, it is important to recognise that rare forms of beta-thalassaemia may be associated with normal HbA₂ levels (Weatherall and Clegg 1981). A more common error may be related to the prevalence of HbA₂', also known as HbB2, resulting from the mutation $\delta 16(A13)Gly \rightarrow Arg$ (GGC>CGC) which occurs in 3–4% of Jamaicans with an AA phenotype (unpublished data) and has been reported

in up to 12% in some West African populations (Bennani et al. 2003). This mutation results in two peaks of HbA₂, one of which may be overlooked (Phylipsen et al. 2010) but both bands are clearly visible on HPLC and should not be missed in experienced laboratories. Since there is an inverse relationship between HbF and HbA₂ levels, high levels of HbF may occasionally lower the HbA₂ values into the normal range. This potential error, which is only significant with HbF levels >10%, may be avoided by calculating the corrected HbA₂ as a proportion of total adult haemoglobin (corrected HbA₂=proportional HbA₂/100-HbF).

It could be argued that since beta-thalassaemia genes occur in less than 1% of this population, attempts to

Table 3 Sensitivity, specificity and area under the ROC curve of seven discriminant functions in 77 subjects with beta-thalassaemia trait and 80 subjects with iron deficiency

Reference	Function	Beta-thal identified	Iron-deficient identified	Sensitivity Pr (True beta-thal)	Specificity Pr (True iron-deficiency)	Area under the ROC curve
England and Fraser 1973	MCV – RBC – (5 × Hb) – 8.4	42/77	75/80	54.5% (42.8–65.9)	93.8% (86.0–97.9)	0.741 (0.679–0.803)
Mentzer 1973	MCV/RBC	38/77	63/80	49.4% (37.8–61.0)	78.8% (68.2–87.1)	0.641 (0.568–0.713)
Shine and Lal 1977	(MCV ² × MCH)/100	68/77	4/80	88.3% (79.0–94.5)	5.0% (1.4–12.3)	0.467 (0.423–0.510)
Green and King 1989	(MCV ² × RDW)/Hb × 100	67/77	76/80	87.0% (77.4–93.6)	95.0% (87.7–98.6)	0.910 (0.865–0.955)
Jayabose et al. 1999	MCV × (RDW/RBC)	55/77	76/80	71.4% (60.0–81.2)	95.0% (87.7–98.6)	0.832 (0.776–0.888)
Srivastava and Bevington 1973	MCH/RBC	44/77	46/80	57.1% (45.4–68.4)	57.5% (45.9–68.5)	0.573 (0.495–0.651)
Ricerca et al. 1987	RDW/RBC	69/77	77/80	89.6% (80.6–95.4)	96.3% (89.4–99.2)	0.929 (0.889–0.969)

ROC (Receiver Operating Characteristic) is an index summarising the accuracy of both sensitivity and specificity in the prediction with perfect sensitivity and specificity giving an area under the ROC curve as 1. These data suggest that the best overall indicators were the formulae proposed by Ricerca et al. (1987) and Green and King (1989)

detect them may not be cost-effective. However, these genes do constitute approximately one out of 15 (7%) Jamaican subjects at risk of a child with sickle cell disease and some babies with sickle cell- β^0 thalassaemia may be clinically affected as severely as SS disease, and certainly, within the context of the Manchester Project, assuring a mother with undetected beta-thalassaemia that she is not at risk of a child with sickle cell disease and who commences to have an affected child would be seriously counterproductive. The need for accurate detection of the beta-thalassaemia trait is illustrated in some other communities where higher frequencies of beta-thalassaemia coincide with HbS such as Chhattisgarh in central India where HbS and beta-thalassaemia both occur in 7–9% of the same population (Patra et al. 2011). An interesting option might be to select HPLC as the screening method of choice for detecting both abnormal haemoglobins and HbA₂ levels suggestive of beta-thalassaemia. Careful cost analysis would be required and Jamaican data would not be helpful since equipment maintenance and reagents must be imported into the island and would be more expensive, whereas excellent technical assistance would be less expensive than elsewhere.

Other issues relate to the use of voluntary or mandatory programmes. There was a phase in the USA, shortly after the passage of the Sickle Cell Anemia Control Act in 1972, when several states enacted well-intentioned mandatory screening programmes for abnormal haemoglobins especially HbS. These programmes created many ethical, educational and social problems and have all been repealed. Mandatory premarital screening laws for the detection of abnormal haemoglobin in potential partners, which have been enacted in Bahrain and Saudi Arabia since 2004, have served to increase awareness of sickle cell disease and the reproductive options but although the screening is mandatory, the families may still choose to continue with marriages at risk of affected children, if other factors seem more important. Most observers agree that in other societies, voluntary participation is preferred and may be increased by education and greater awareness. In Jamaica, interest in the programme among the targeted school students has increased steadily, voluntary compliance with testing increasing from 56% to 88% over the first 4 years of the study.

As with all education, repeated reinforcement of the genetic information is necessary. Awareness of the significance of HbS is high among this community and that of HbC is increasing. Beta-thalassaemia, however, is largely unknown and has required special counselling and the development of specific literature. The ultimate objective of the Manchester Project, which is to determine whether knowledge of haemoglobin

genotype will influence reproductive decisions, must await the long-term outcome which will be derived from the results of newborn screening among all the births in southern Jamaica.

In the meantime, and within the context of the Manchester Project, we believe that detection of all genes placing subjects at risk of a child with sickle cell disease should be as complete as possible. Our procedures may be more rigorous than those used in general population screening but this reflects the need for accuracy in addressing the objectives of this project.

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Conflict of interest The authors declare that they have no conflict of interest.

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