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Newborn screening for sickle cell disease in Brazil: the Campinas experience

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Summary Newborn screening for sickle cell disease commenced in 1992 in Sao Paulo State and by the end of 2000, the programme covered 78 institutions in 36 municipalities with the screening of 281 884 babies. Initially based on liquid cord blood samples, these are being replaced by dried filter paper capillary samples to ease handling and avoid diagnostic confusion from maternal contamination. The prevalence of sickle cell trait (2.0%) and HbC trait (0.6%) increased significantly between 1996 and 2000, apparently because of improved detection rather than the later introduction of institutions serving populations with higher trait frequencies. There were 29 babies with homozygous sickle cell SS disease and 26 with sickle cell-haemoglobin C (SC) disease, the latter significantly exceeding expectation and possibly attributable to a nonrandom selection of partners. Sickle cell-β thalassaemia syndromes were proportionately more common than in Jamaica, and it is possible that this results from interaction with other Brazilian populations carrying higher β thalassaemia gene frequencies. The frequency of abnormal haemoglobins in this population is lower than in Jamaica, but clinically significant sickle cell disease occurred once in every 5527 births, comparable with the frequencies of other significant inborn errors of metabolism.

Keywords Sickle cell disease, newborn screening, Brazil, isoelectric focusing, Bantu haplotype

Introduction

The benefit of early diagnosis and intervention in the management of sickle cell disease (Consensus Development Conference, 1987) has led to widespread programmes for the early detection of this condition. Newborn screening is practiced in most of the USA, increasingly in Europe, and a pilot programme is underway in Ghana. In the Caribbean, screening commenced in Jamaica in 1973 to establish the Jamaican Cohort Study (Serjeant *et al.*, 1986) and in St Lucia in 1990. In Brazil, the most comprehensive newborn screening programme started in Minas Gerais in 1998 (Serjeant, 2000), although a programme in Campinas, Sao Paulo State began in 1992 and has proceeded more slowly. The latter programme is reviewed, as the experience may be relevant to the design of screening in developing societies.

Subjects and methods

The programme is based at the University at Campinas, a city of approximately 1 million people, 120 km from the State capital, Sao Paulo. There are approximately 38 million people in the State of Sao Paulo, of whom nearly half live in the city of Sao Paulo. The population derives from many ethnic and national origins and includes the native American Indian people, Europeans of Portuguese, Italian and German origin, Japanese and African people, the latter representing up to 30% of some urban populations. The screening programme was initiated at a maternity hospital close to Campinas in 1992, extended to a major maternity hospital in the city in 1994 and to other local hospitals from 1996. By the end of 2000, the programme served 78 institutions in 36 cities including four hospitals in Sao Paulo, a city with an estimated 180 000 deliveries annually.

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Samples

The service initially used liquid cord blood samples transported in refrigerated containers by road to the central laboratory at the Centro Integrado de Pesquisas Oncohematologicas na Infancia (CIPOI) at the University of Campinas (Unicamp). Difficulties in handling liquid samples and the occasional diagnostic confusion from maternal contamination has led to increasing use of filter paper cards and to heel prick samples taken at ages 5–7 days, when the child attends hospital for follow-up.

Electrophoretic methods

Haemoglobin electrophoresis was initially performed on cellulose acetate with confirmation of abnormalities on agar gel but in April 2000, this was replaced by isoelectric focusing gels (Kutlar *et al.*, 1990), analysed by the RESOLVE Neonatal Haemoglobin Test Kit system (Wallac, Wallac Inc., Akran, OH, USA), which identified haemoglobin bands by their isoelectric point and was capable of detecting HbS and HbC and also some of the less common variants such as HbD_{Punjab}, $HbG_{Philadelphia}$ and HbE.

Methods of follow-up

The families of all babies with haemoglobin variants were requested to attend CIPOI for counselling, conducted by two specialist nurses who perform 1250 counselling sessions each year. Babies with sickle cell disease [homozygous SS disease, sickle cell-haemoglobin C (SC) disease, sickle cell-β° thalassaemia and sickle cell-β^{+} thalassaemia] were referred for follow-up at the Centro Infantil Boldrini, a major paediatric haematology facility close to the Unicamp campus.

Statistical methods

The frequency of the HbS and HbC traits were described as annual incidence rates with 95% jackknife confidence intervals. Secular trends were tested by a log-linear test for trend independently for the two traits. The apparent increase in HbS and HbC trait frequency was further explored using a random-effects log-linear model, which allowed for the longitudinal nature of the data and the differential effects of individual hospitals. Whether the observed frequencies of SS and of SC disease deviated from that predicted by the Hardy–Weinberg equilibrium was tested by Pearson's chi-squared test.

Results

Population screened

A total of 281 884 babies were screened between August 28, 1992 and December 31, 2000, the annual totals increasing with the number of institutions (Table 1). Major increases occurred with the addition of the Maternidade de Campinas (over 10 000 babies annually) in 1994, three institutions (together providing over 7000 babies) in 1996, two with over 4000 babies in 1997 and five institutions in Sao Paulo with over 15 000 deliveries annually in 2000. The latter institutions were added in late 2000, so their full impact is yet to be seen.

Frequency of haemoglobin variants

The frequency of the sickle cell trait varied between towns and institutions within the same town. Overall, between 1992 and 2000, there was a secular increase in the frequency of the sickle cell trait (rate ratio 1.07, 95% CI 1.05–1.09, $\chi^2 = 83.3$, $P < 0.001$) (Figure 1) and the HbC

Table 1. Frequencies of HbS and HbC traits since onset of programme

Year	Institutions screened*	Population screened	Sickle cell trait			HbC trait		
			n observed	incidence	95% CI	*n* observed	incidence	95% CI
1992	1	379	8	2.11	1.09–4.69	3	0.79	0.25–3.86
1993	1	1110	20	1.80	1.18–2.88	6	0.54	0.25–1.42
1994	2	9702	150	1.55	1.32–1.82	57	0.59	0.46–0.77
1995	3	11713	200	1.71	1.49–1.96	55	0.47	0.36–0.62
1996	15	22563	336	1.49	1.34–1.66	119	0.53	0.44–0.63
1997	65	40838	567	1.39	1.28–1.51	216	0.53	0.46–0.61
1998	67	62632	1126	1.80	1.70–1.91	367	0.59	0.53–0.65
1999	68	62995	1214	1.93	1.82–2.04	369	0.59	0.53–0.65
2000	78	69952	1576	2.25	2.15–2.37	423	0.60	0.55–0.67

*Denotes year in which screening started in each year and usually only reflects part of year.

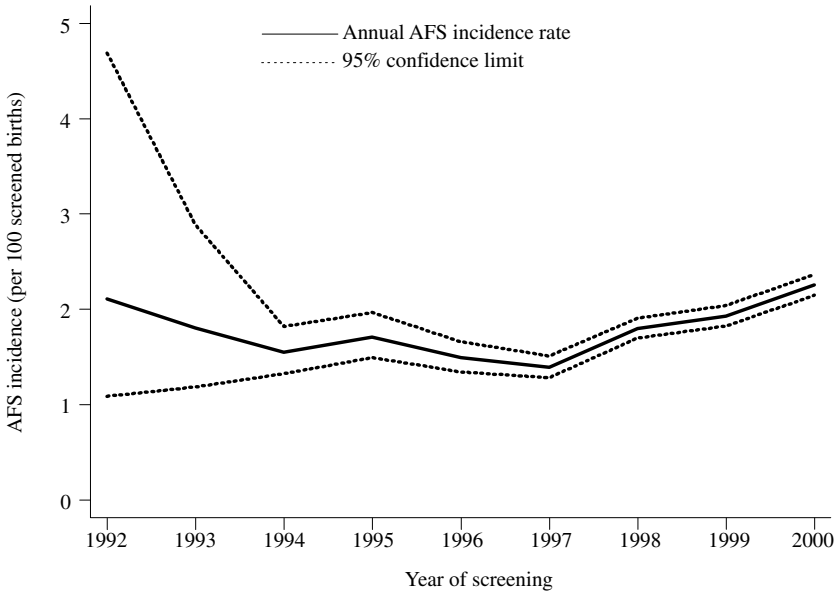

Figure 1. The annual incidence in frequency of the sickle cell trait illustrating the secular increase.

trait (rate ratio 1.07, CI 1.05–1.08, $\chi^2 = 80.2$, $P < 0.001$). As this secular trend could have resulted from increasing sensitivity of laboratory detection or the addition of hospitals with higher trait rates, the increasing HbS trait frequency was further explored to distinguish these possibilities. The unadjusted secular increase in HbS trait frequency between successive years of screening was approximately 10% per year (rate ratio 1.10, 95% CI 1.08–1.12, $P < 0.001$), which remained unchanged after adjusting for year of entry into the screening programme. Only hospitals recruited in 2000 had a marginally increased HbS trait frequency (rate ratio 2.00, 95% CI 1.01–3.96, $P = 0.05$). Predicted HbS trait frequencies stratified by year of admission of hospitals to the programme (Figure 2) confirmed that an apparent increase occurred within institutions and could not be accounted for by the addition of new institutions with higher frequencies. Greater sensitivity in detection following the introduction of isoelectric focusing in April 2000 was not supported by an apparent step increase in frequency when the individual months of 2000 were compared and the most likely interpretation of this secular trend appears to be a generally increasing sensitivity of detection of the HbS trait.

The frequencies of principle genotypes are summarized in Table 2. The gene frequency calculated by gene counting was 0.00938 for the S gene and 0.00292 for the HbC gene giving an expected yield of 24.7 SS and 15.4 SC babies compared with observed numbers of 29 and 26, respectively. The observed frequency of major genotypes deviated from that expected from the Hardy–Weinberg

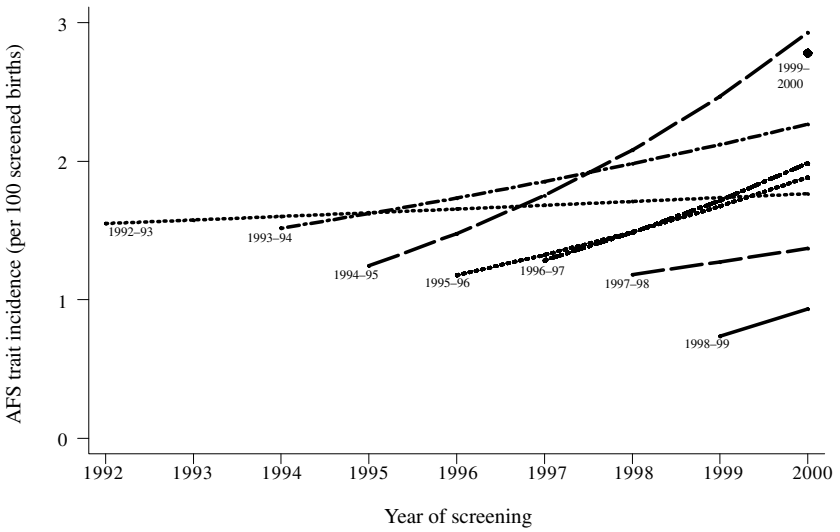

Figure 2. Predicted frequencies of the sickle cell trait for the years 1992–2000 among 78 hospitals stratified by year of entry into the screening programme, allowing for differential longitudinal patterns.

Table 2. Frequency of major haemoglobin phenotypes among 281 884 samples between 1992 and 2000

Sickle cell trait	AFS	5197 (1.98%)
HbC trait	AFC	1615 (0.57%)
Homozygous sickle cell disease	SS disease	29
Sickle cell-haemoglobin C disease	SC disease	26
Sickle cell-β° thalassaemia	Sβ° thal.	2
Sickle cell-β^+ thalassaemia	Sβ$^+$ thal.	4
Sickle cell-hereditary persistence of foetal haemoglobin	S/HPFH	1
Homozygous HbC disease	CC disease	1
HbC-β° thalassaemia	C-β° thal.	2
HbC-β^+ thalassaemia	C-β$^+$ thal.	1

equation ($\chi^2 = 8.97$, df = 3; $P = 0.03$), 81.5% of this deviation being accounted for by the excess of SC disease and only 8.2% resulting from the apparent excess of SS cases (Table 3). Sickle cell-β° thalassaemia was not always distinguished from sickle cell-β^+ thalassaemia, so formal testing of the respective β thalassaemia gene frequencies was not possible but there was a suggestion that they may have been relatively more common than in Jamaican populations.

Discussion

Brazil occupies over half of the land mass of South America with a population of 160 million drawn from European, Asian and African populations in addition to the indigenous Indian people. The sickle cell gene in Brazil is believed to derive predominantly from the African population although genetic admixture over 300 years imply that many carrying the gene do not have African features. Combinations of the sickle cell gene with the β thalassaemia genes of Italian origin further reduces the specific racial features of some patients with sickle cell-β thalassaemia syndromes.

The African population of Brazil derived essentially from the former Portuguese colonies in Africa, 0.4 million people from Costa da Mina and Guinea Bissau going to Recife in Pernambuco State, 1.2 million from the Benin Gold Coast to Salvador and 2.0 million from Congo and Angola to Rio. In 1817 the total population of Brazil was 3.6 million of whom 1.9 million were African slaves, the historical record showing that 100 000 slaves were imported in the 16th century, 600 000 in the 17th century, 1.3 million in the 18th and 1.6 million in the 19th century (Verger, 1968; Curtin, 1969). From this initial distribution, a large population of African origin remains in the States of Bahia and Minas Gerais but there has been a recent substantial movement of people to the urban areas Rio de Janeiro and Sao Paulo.

These observations are relevant to the Campinas programme in which frequencies of the sickle cell trait averaged 2.0% but exceeded 3% in the urban areas of Sao Paulo. However, the HbC trait, a marker of West African ancestry, occurred in 0.6% and implies that part of the population derived from West Africa rather than the Congo and Angola which was believed to supply most of the African population in Rio de Janeiro.

Calculated on the basis of gene frequency, there was an excess of patients with SC disease, the most likely interpretation of which may be a nonrandom association of partners. The six cases of sickle cell-β thalassaemia had not all been assigned to β^+ or β° types so formal testing of their gene frequencies was not possible and would have led to minor inaccuracies of the model presented in Table 3. There was an impression that sickle cell-β thalassaemia syndromes were more common than in Jamaica, the relative ratio of SS to sickle cell-β thalassaemia of all types being 4.8 : 1 in Campinas compared with 6.7 : 1 in Jamaica.

Compared with Jamaica, Sao Paulo State manifests lower frequencies of abnormal haemoglobins, a case of SS disease occurring once in every 9720 babies compared with every 300 babies in Jamaica. However, clinically significant forms of SS disease occur once in every 5527 births in the Sao Paulo programme comparable with the frequencies of other significant inborn errors of metabolism such as hypothyroidism (1 : 3500 births) and phenylketonuria (1 : 12 000 births) (Zago *et al.*, 1983).

Brazil also offers a research opportunity for comparing the manifestations of Benin and Bantu haplotypes. The

Table 3. Observed and expected genotype frequencies for the β^A, β^S and β^C alleles, assuming Hardy–Weinberg equilibrium

	AA	AS	AC	SS	SC	CC	Total
Observed (O)	275 016	5197	1615	29	26	1	281 884
Expected (E)	275 002.5	5216.14	1622.82	24.73	15.39	2.39	281 884
$(O - E)^2/E$	0.0007	0.0702	0.0377	0.7360	7.3147	0.8118	8.97

$\chi^2 = 8.97$, df = 3, $P = 0.03$.
AA, normal haemoglobin genotype; AS, sickle cell trait; AC, HbC trait; SS, homozygous sickle cell disease; SC, sickle cell-haemoglobin C disease; CC, homozygous HbC disease.

Benin haplotype predominates among African Americans and in the Caribbean where 60% of SS patients are homozygous for the Benin haplotype and a further 20% heterozygotes (G.J. Dover and G.R. Serjeant, unpublished observations) whereas Bantu represent <10%. In Brazil, the Bantu haplotype accounts for 55–73% and the Benin 25–45% (Figueiredo *et al.*, 1994) affording an opportunity to compare the expression of the two haplotypes within the same environment.

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