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SCREENING FOR INHERITED METABOLIC DISEASES AND CONGENITAL HYPOTHYROIDISM IN 4,744 MENTALLY RETARDED SCHOOL CHILDREN IN TAIWAN

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Summary For the purpose of exploring the possibility of implementing a nation-wide screening program for inherited metabolic diseases and congenital hypothyroidism in Chinese newborn infants, a pilot study was initiated in 1983 to detect patients with phenylketonuria (PKU), galactosemia, homocystinuria, maple syrup urine disease and congenital hypothyroidism (CHT) in mildly mentally retarded (mostly IQ 50–75) school children in Taiwan. Of 4,744 blood samples collected on filter paper from 246 primary and junior high schools all over the island, preliminary screening disclosed six suspected positive cases of PKU and nine of CHT. Two cases of classical PKU, one case of atypical PKU caused by tetrahydrobiopterin deficiency, and seven cases of CHT (one hypoplastic, two athyroid, three ectopic and one dyshormonogenesis) were finally confirmed. In addition to the seven cases of CHT, one more case found by questionnaire survey. This case was missed from the screening because he was recognized as CHT previously and was on thyroxine replacement therapy during screening. The incidence is 1/1,581 for PKU and 1/593 for CHT in these children.

INTRODUCTION

Although neonatal screening for metabolic diseases has become routine preventive pediatrics in some of the developed countries (Bickel, 1980), no such measure on a nationwide scale has been initiated with the Chinese newborn in Taiwan. Basic information such as the incidence of phenylketonuria or other metabolic diseases is lacking. With the aim of establishing a nationally neonatal screening program

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in Taiwn, we first in 1983 initiated a pilot screening study on all mentally retarded children who were registered in special classes of primary and junior high schools. The rationale for doing this is the following: (1) The mental retardates are likely "reservoirs" of those hereditary diseases which are of screening importance. Pilot screening of such high-risk groups for these diseases, before their inclusion in screening program of the general population, is a helpful source of incidence data on which to assess the merits of wider surveys. (2) By screening the mentally retarded school children, we do not have to worry too much about our inexperience in assays which might result in false positives or false negatives. Since the mental condition of these children is too late to be improved by any diet therapy or drug treatment, there is no urgency for proper management or evaluation of any positive cases found thereof. (3) Methods for confirmatory diagnosis and clinical management could be established by follow-up studies of the positive cases found in these children.

This report describes the screening of 4,744 mentally retarded school children for phenylketonuria, galactosemia, homocystinuria, maple syrup urine disease, and congenital hypothyroidism with the intent of applying the information obtained and the methodologies established to lay foundations for neonatal screening.

MATERIALS AND METHODS

Blood samples

Blood samples from 4,744 mentally retarded children registered in 448 special classes of 246 primary and junior high schools all over the island were collected on filter paper (Toyo Roshi, Ltd., Tokyo, Japan) by local public health nurses and sent by mail to the screening center at Clinical Biochemistry Research Laboratory, Department of Medical Research, Veterans General Hospital in Taipei for assay. The children were aged 6–20; most of them had IQ 50–75.

Screening assays

1) Bacterial inhibition assay (BIA) for phenylketonuria, homocystinuria and maple syrup urine disease. BIA devised by Guthrie and Susi (1963) was applied. *B. subtilis* ATCC 6633 (10^7/ml) and modified Demain's Minimum Medium were provided by Eiken Chemical Co., Ltd., Japan. Other reagents included agar (Difco Lab., USA); standard blood strips with known concentration of respective amino acid (Fujirabio Inc., Japan); antimetabolites of β-2-thienylalanine, 4-azaleucine and methionine sulfoximine (Eiken Co., Ltd., Japan). Three mm discs of the autoclaved dried blood spots and the standards were punched out and incubated on the agar plates at 37°C and 60% relative humidity for 16–18 hr. Bacterial growth was measured by the diameter of the growing zone around the blood spot. Concentration of phenylalanine, leucine or methionine in the blood was calculated from the corresponding standard curves. Values equivalent to or above the following were considered abnormal: phenylalanine, 2 mg/dl; leucine, 2 mg/dl; methionine, 1 mg/

dl. Repeated assays on the original blood spots as well as on the second blood samples of suspected cases were carried out.

2) Modified Paigen's test for galactosemia. Paigen *et al.* (1982) developed a bacteriophage assay to detect the presence of galactose in blood. Modified Paigen's modium, *E. coli* Q396 ($1–2 \times 10^{10}$/ml), phage C21 ($1–3 \times 10^{8}$/ml) were all provided by Eiken Chemical Co., Ltd., Japan. Other reagents were agar No. 1 (Oxoid Ltd., U.K.), glycerin (Sigma, USA), and 2,3,5-triphenyl-tetrazolium chloride (Sigma, USA). The culture condition and the way of determining the blood galactose concentration were similar to those of the BIA assay except that unautoclaved blood spots were used. Blood galactose 4 mg/dl or higher was considered abnormal. Further tests were performed on all suspected positive cases.

3) Enzyme immunoassay (EIA) of thyrotropin (TSH) for congenital hypothyroidism. Enzyme immunoassay for TSH (Naruse *et al.*, 1982) was used. The following reagents were provided by Fujirabio Inc., Japan: Anti-human TSH antiserum, β-D-galactosidase-TSH conjugate, polyacetol bead coated with anti-rabbit IgG, 4-methyl umbelliferone-β-D-galactoside solution (6.3 mg/ml), standard blood spots with 0, 10, 20, 40, 80, 160 μU TSH/ml blood. Standard and duplicated dried blood spots with a diameter of 4.25 mm punched from filter paper were used for this assay. Blood TSH 10 μU/ml or higher was taken as abnormal.

Confirmatory tests

1) Phenylketonuria. All children with consistent findings of higher than normal blood phenylalanine level were subjected to a comprehensive physical examination. Ferric chloride test (Shih, 1973), flourometric determination of serum phenylalanine (McCaman and Robins, 1962) and tyrosine (Ambrose, 1977), quantitative assay of urinary metabolites by gas chromatography (Hsiao *et al.*, 1985) and the measurement of blood Phe/Tyr ratio by amino acid analyzer (Mondine *et al.*, 1972) were performed. Atypical PKU caused by tetrahydrobiopterin deficiency was diagnosed by analysis of urinary pterins by high performance liquid chromatography (Liu *et al.*, 1985; Niederwieser *et al.*, 1982).

2) Congenital hypothyroidism. In addition to clinical evaluation, serum thyrotropin (TSH), thyroxine (T4) and triiodothyronine (T3) were determined by radioimmunoassay (Naruse *et al.*, 1982). Thyroid scanning by technetium-99m or iodine-131, bone age measurement by X-ray, and determination of serum titers of antibodies against thyroid (antithyroid antibody and antimicrosomal antibody) were performed to check the etiopathogenesis of hypothyroidism.

Quality assurance

In order to assure the quality of our assays, we studied the following parameters: stability of the dried blood in storage, sensitivity, linearity, accuracy, and precision of BIA, EIA and phage assay. Quality control samples were included in every analytical run and the acceptance of the test results was determined according to Westgard's Multi-Rule (Westgard *et al.*, 1983). The details of these preliminary

data have been reported elsewhere (Wuu and Hsiao, 1983 and 1984).

A questionnaire survey of every case was conducted during blood sample collection to study the possibility of false negative results in our screening tests.

RESULTS

This survey covered all the public schools which have special classes for mental retardates in Taiwan. The number of students registered was 4,953 and the number of blood samples collected was 4,744 which represented a 95% collection rate.

After preliminary screening test of the 4,744 blood samples, the number of children with values above normal was as following: six for phenylketonuria, none for galactosemia, nine for congenital hypothyroidism, none for homocystinuria and nine for maple syrup urine disease (Table 1). Second blood samples were taken from all suspected positive cases with the exception of one PKU suspect (Phe 2–5 mg/dl) who moved with her family to an unknown address. None of the children was found to be galactosemia, homocystinuria or maple syrup urine disease. All second samples of the nine suspected cases of congenital hypothyroidism were still positive. Only three of the five second samples of suspected PKU positive cases were still politive. All of them had blood phenylalanine higher then 8 mg/dl in the first screening sample. Comprehensive clinical and biochemical tests were carried out for all positive cases confirmed by the recalled sample. Three cases of PKU, two classical and one atypical (tetrahydrobiopterin synthesis deficiency), and

Table 1. Screening results of 4,744 mentally retarded school children.

Disease	Concentration in blood spot	Number of positive cases
Phenylketonuria	2–5 mg Phe/dl	3
	5–8	0
	8–10	1
	12–14	1
	14–20	1
Homocystinuria	1–3 mg Met/dl	0
Maple syrup urine disease	2–4 mg Leu/dl	9 (2–4 mg/dl)[a]
Galactosemia	4 mg Gal/dl	0
Congenital hypothyroidism	10–30 μU TSH/ml	4
	31–50	3
	70–90	2

[a] All nine cases were false positive due to poor quality of the blood sample.

Table 2. Results of confirmatory tests of the three PKU children.

Case No.	Sex	Age	IQ (DQ)	$FeCl_3$ test	GC [a]	Serum conc. (mg/dl)		Phe/Tyr	B% [b]	Diagnosis
						Phe	Tyr			
P1	M	13	75	+	+	13.6	1.3	10	0.9	Atypical PKU (tetrahydrobiopterin synthesis deficiency)
P2	M	16	69	+	+	26.7	1.1	24	74.6	Classical PKU
P3	F	11	37	+	+	33.1	0.8	41	65.2	Classical PKU

[a] Gas chromatography. [b] Urinary total biopterin ratio (%)=biopterin/(biopterin+neopterin).

Table 3. Clinical data of nine mentally retarded school children suspected as congenital hypothyroidism. Seven of them were confirmed. One more CHT was picked up by questionnaire survey rather than by screening (see text).

Case No.	Sex	Age (year/month)	Height (cm)	Weight (kg)	Bone age (year)	IQ	Thyroid scan	Diagnosis
T1	M	19/9	159.5	57.5	Normal	63	Hypoplastic	CHT
T2	M	12/3	121.5	23.5	6	18	Athyroid	CHT
T3	F	12/8	121.0	24.8	8	53	Ectopic	CHT
T4	F	9/6	126.0	23.0	4.5	55	Ectopic	CHT
T5	M	11/10	124.0	26.2	7	30	Ectopic	CHT
T6	M	18/10	136.0	31.0	14	25	Athyroid	CHT
T7	M	15/10	125.5	27.0	9	43	Slightly enlarged [a]	CHT (Dyshormonogenesis)
T8	F	13/3	135.0	44.3	11	54	Normal	Trisomy 21 with autoimmune thyroiditis [b]
T9	M	13/6	161.5	44.5	13	74	Normal	Atypical PKU (=P1)

[a] The thyroid uptake of ^{131}I at 24 hr was 68% (normal range: 15–40%). [b] Antimicrosomal antibody positive (1 : 1,600).

seven cases of congenital hypothyroidism were observed. The clinical and biochemical findings are shown in Tables 2–4.

In addition to the seven cases of CHT listed in Table 3, one case was missed from the initial screening because he was recognized as CHT by a pediatrician before screening and was on thyroid tablet treatment during screening. He was picked up by questionnaire survey. Patients T1, T2 and T6 were also diagnosed previously and were on thyroid tablet treatment. But inadequate dosage caused positive results in our screening test. One (P3) of three PKU cases was recongnized at the age of

Table 4. Biochemical data of nine mentally retarded school children suspected as congenital hypothyroidism. Seven of them (T1–T7) were confirmed.

Case No.	Blood spot TSH concentration (μU/ml)		Serum concentration		
	Initial screening	Recalled test	TSH (μU/ml)	T3 (ng/dl)	T4 (μg/dl)
T1	13.0	<10 [a]	55	118	2.2
T2	31.5	42.0	>80	78	2.5
T3	123.0	66.5	404	97	1.3
T4	35.5	43.5	220	128	4.7
T5	16.0	29.5	90	125	4.7
T6	11.5	31.0	89	126	2.4
T7	35.0	41.0	137	77	0.7
T8	65.0	60.0	344	102	1.2
T9	13.5 [b]	<10	0.7	176	11.3
Normal range	<10	<10	<6.2	85–165	6.0–12.0

[a] This patient was on thyroid tablet when the second blood sample was taken. [b] This patient was on antithyroid drug when the first blood sample was taken.

one year. No other case was identified by the questionnaire survey. Contrarily, patient T9 was misdiagnosed by a doctor as hyperthyroidism and was on antithyroid drug, which caused the hypofunction of the thyroid, when the first blood spot sample was taken. He was confirmed later as atypical PKU (P1 of Table 2). Patient T8 has Down's syndrome (trisomy 21) with hypothyroidism caused by auto-immune thyroiditis (antimicrosomal antibody tests were positive). These results indicate that our screening assay was sensitive and reliable.

The incidence of PKU and congenital hypothyroidism of these mentally retarded school children was 6.32 and 16.86 in 10,000, respectively. Two of the three PKU's and six of the nine congenital hypothyroidism patients were male. Their ages were from nine to 20 with IQ 18–75. Of these eight hypothyroidism patients, the thyroid scanning showed that one was hypoplastic, two athyrotic, three ectopic, one slightly enlarged (with increased iodine uptake by the thyroid) and one normal (Table 3). Follow up studies and genetic counseling were offered to the family members of the PKU patients and the case of CHT caused by dyshormonogenesis.

DISCUSSION

The etiology of mental retardation is often difficult to determine. It has been estimated that about 5% of severely retarded cases, not including Down's syndrome, involve a hereditary component (Milunsky, 1975).

In the present report, we found three cases of phenylketonuria and eight cases of congenital hypothyroidism among 4,744 mentally retarded school children. There

are two features of this study which differ from most of the cited reports. First, the children are not severely mentally retarded. Most of them had an IQ of 50–75. Second, the scope of screening was limited to those few diseases which are of neonatal importance. For these reasons, the low incidence of metabolic diseases found in this selected group of children is not unexpected.

The case of atypical phenylketonuria caused by tetrahydrobiopterin synthesis deficiency may be a partial or a peripheral form as described by Dhondt (1984). One out of three PKU in the present study was caused by tetrahydrobiopterin deficiency, which was reported to be rare (1% to 3% among PKU) in Caucasians (Dhondt, 1984). It is interesting to study further whether Chinese have a higher frequency of tetrahydrobiopterin deficiency. Bone ages of all CHT cases found in this study were delayed, except the hypoplastic case (T1) which has been diagnosed and treated since age of 7 years old. The mental deficiency was more severe in the two athyrotic children (T2 and T6), even though they had been diagnosed and treated since age of 6 months and 10 years old, respectively, than in children with ectopic thyroids (Table 3). These were consistent with previous reports in other populations (Burrow *et al.*, 1980).

Our main goal is the prevention of mental retardation through neonatal screening of the general population. Although the modified bacteriophage assay for galactose and the enzyme immunoassay for TSH have not been widely used in other screening programs, these screening assays proved to be sensitive and reliable in our hands. With all the experiences gained from and systems established in this pilot screening study, a nationally supported newborn screening program is now ongoing.

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