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Inherited Metabolic Disorders in Turkey

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Summary: A high prevalence of inherited metabolic diseases is present in Turkey; at least in part, this is due to consanguineous marriages.

The frequencies of mutant genes show remarkable variations from one country to another. In Turkey, marriages between relatives are relatively common and the frequency of autosomal recessive conditions would be expected to be high (Tunçbilek and Ulusoy, 1989). A survey has therefore been carried out to determine the incidence of phenylketonuria (PKU) in order to assess the magnitude of the problem and to gain experience in screening organization in Turkey. Moreover, a group of selected infants and children with motor and mental retardation (MMR) were also systematically investigated to document the prevalence of various inborn errors of metabolism as compared to PKU. This might lead us to a prenatal diagnostic approach to the diseases with the highest prevalence. We report here the results of this survey which give a picture of a developing partly Asian partly European country.

MATERIAL AND METHODS

The survey was carried out on the following groups of children:

(a) High risk newborns and infants
(b) Children with MMR
(c) Elementary school students (for cystinuria)
(d) Normal newborns (for PKU)

Symptoms classifying infants as being at high risk were failure to feed, persistent vomiting, seizures, lethargy, coma, hypotonia/hypertonia, developmental delay and regression of developmental milestones, acidosis, persistent jaundice, unusual urine odour, failure to thrive, cataracts, and a history of a previous child with one of the inherited metabolic diseases.

Systematic screening procedures were applied to selected infants. Their blood and urine amino acids were investigated by one or two-dimensional paper chromatography (Shih, 1973). In addition to paper chromatographic procedures, some clinical

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diagnostic tests were performed on each urine sample (Shih, 1973). Blood gases, total CO_2 content, ammonia, electrolytes and glucose were also determined in certain cases. Quantitative laboratory methods were performed when possible on the patients with abnormal results for confirmation of the diagnosis. Organic acid analysis was performed by GC-MS. Plasma and urine amino acids were determined by ion exchange column chromatographic procedures (Biotronic and LKB amino acid analysers). CSF amino acids were also studied in patients with suspected non-ketotic hyperglycinaemia (NKH). Cases with high CSF glycine concentrations and high CSF glycine/plasma glycine ratios were considered to have NKH (Perry *et al.*, 1975). Slit lamp examinations of eyes were carried out in cases with generalized amino aciduria and with other tubular dysfunctions, and diagnosis of cystinosis was confirmed by the presence of cystine crystals in the patient's cornea. For the classification of hyperammonaemias, plasma and urine amino acid patterns and daily orotic acid excretions of the patients were taken into account. Diagnosis of galactosaemia was confirmed by the determination of galactose-1-phosphate uridyl transferase (EC 2.7.7.12) activity in erythrocytes (Beutler and Baluda, 1966).

Children with MMR had been screened mainly for hereditary amino acidopathies. For the screening of cystinuria, a sodium cyanide nitroprusside test was performed on urine samples from 8072 elementary school students. Two-dimensional paper chromatography and quantitative cystine determinations were made in children with positive results. Children who excreted $> 300\,mg\,day^{-1}$ cystine were diagnosed as having cystinuria. Normal newborns were screened for PKU by the Guthrie test (Guthrie and Susi, 1963). Infants with a serum phenylalanine level exceeding $20\,mg\,dl^{-1}$ by the fluorometric method (McCaman and Robins, 1962) and with urine giving a positive reaction with ferric chloride either initially or at the time of a protein challenge later in infancy were regarded as having classical PKU. Infants whose serum phenylalanine levels were persistently higher than $8–10\,mg\,dl^{-1}$, but who did not meet the above-mentioned criteria were classified as having hyperphenylalaninaemia.

RESULTS AND DISCUSSION

Results for selected infants

A total of 225 cases with 21 different hereditary metabolic diseases were detected in 6050 selected infants (3.75%). The figure that we obtained for the prevalence of metabolic diseases is higher than those reported from some other countries (Chalmers *et al.*, 1980; Krieger *et al.*, 1982). PKU, maple syrup urine disease (MSUD), methylmalonic acidaemia, hereditary urea cycle defects and galactosaemia were the most commonly seen metabolic diseases in this group (Table 1).

It has been indicated that defects in amino acid metabolism and hereditary organic acidaemias can be detected by the first line screening procedures which were also performed in our patients (Stöckler *et al.*, 1988). Blood and urine amino acids of each infant were investigated by paper chromatography and quantitative amino acid

Table 1 Prevalence of inborn errors of metabolism in 6050 high-risk infants for metabolic disease

Metabolic disease	*No. of cases*	*%*
Phenylketonuria	116	1.91
Alcaptonuria	7	0.11
Tyrosinaemia type I	1	0.01
Homocystinuria	2	0.03
Cystinosis	7	0.11
Cystinuria	6	0.09
Maple syrup urine disease[a]	20	0.33
Prolinaemia	1	0.01
Iminoglycinuria	1	0.01
Non-ketotic hyperglycinaemia	3	0.04
Hyperammonaemias	10	0.16
OTC deficiency	3	0.04
Citrullinaemia	6	0.09
Lysinuric protein intolerance	1	0.01
Organic acidaemias	31	0.51
Methylmalonic acidaemia	13	0.21
Propionic acidaemia	5	0.08
Isovaleric acidaemia	1	0.01
β-ketothiolase deficiency	1	0.01
Lactic acidaemia[b]	3	0.04
Glutaric aciduria type II	1	0.01
Pyroglutamic aciduria	1	0.01
Organic acidaemia (unknown)	6	0.09
Fructose intolerance[c]	6	0.09
Galactosaemia	16	0.26
Total	227	3.75

[a]Classical maple syrup urine disease
[b]3 cases with pyruvate dehydrogenase deficiency, 2 with mitochondrial myopathy, 1 not categorized
[c]In 3 cases, clinical and laboratory findings were compatible with fructose-1,6-diphosphatase deficiency
For standard references on these conditions see McKusick catalogue

studies were performed on infants with abnormal results. We therefore think that all cases with hereditary abnormal amino acid metabolism could be detected. However, since the urinary organic acid profiles were investigated only in cases with persistent metabolic acidosis, hereditary organic acidaemias such as multiple acyl-CoA dehydrogenase deficiency and γ-hydroxybutyric acidaemias which had not presented clinically with metabolic acidosis might have been missed.

In four newborns who were included in the survey due to persistent vomiting, laboratory findings were found to be compatible with PKU and vomiting in these patients was overcome with a phenylalanine restricted diet. These observations indicate that vomiting is not a rare sign of PKU. In fact, this is one of the metabolic diseases in which pyloromyotomy has been carried out because of misdiagnosis of pyloric stenosis (Nyhan, 1980; Burton, 1987). Except for 30 infants who were the siblings of PKU patients, the other phenylketonuric infants have been brought to

the hospital due to either MMR or convulsions. There was parental consanguinity in 72% of the 225 detected cases, which is three times higher than the prevalence of consanguineous marriages in Turkey at present.

Screening results for children with MMR

A total of 572 cases with 12 different types of amino acidopathies were detected in 10 800 children with MMR. Of these children, 510 who were referred to Hacettepe Children's Hospital for further investigations have been diagnosed as having PKU (4.7%) (Table 2). The prevalence of PKU in this selected population is almost twice as high as that observed in Ireland where the incidence of PKU is highest among the reported surveys (Moore *et al.*, 1972). The parents of 45% of these cases were relatives. In more than half of the families, however, there was no consanguinity. This could indicate a high frequency of mutations in the phenylalanine hydroxylase gene in Turkey.

As far as the distribution of PKU in Turkey is concerned, the cases are found in all parts of the country. The higher PKU prevalence of hereditary amino acidopathies in certain regions of the country reflected a biased referral pattern (Figure 1).

Twenty-two patients (0.2%) with homocystinuria were detected and the prevalence of hereditary aminoacidopathies was found to be as high as 5.3% in this selected population (Table 2).

Screening of normal children for cystinuria

Cystinuria is an inherited condition affecting the active transport of the diamino acids cystine, ornithine, lysine and arginine across the renal tubule and the small intestine. The only clinical effect is the production of urinary tract stones in some of the cases. In Turkey, urolithiasis is common (Eckstein, 1961). However, we know neither the incidence of cystinuria nor the prevalence of urolithiasis due to cystinuria.

Table 2 The prevalence of PKU, homocystinuria and other aminoacidopathies in 10 800 children with mental-motor retardation

Metabolic disease	*No. of cases*	*%*
Phenylketonuria	510	4.7
Homocystinuria	22	0.2
Others[a]	40	0.4
Total	572	5.3

[a]Number in parentheses indicates the number of cases detected: cystinuria (12); Hartnup disease (7); citrullinaemia (4); maple syrup urine disease (intermediate type) (3); prolinaemia (3); iminoglycinuria (3); alaninaemia with lactic acidosis (pyruvate dehydrogenase deficiency) (2); cystathioninuria (3); hyperglycinaemia (persistent, unclassified) (2); hyperlysinaemia (1)

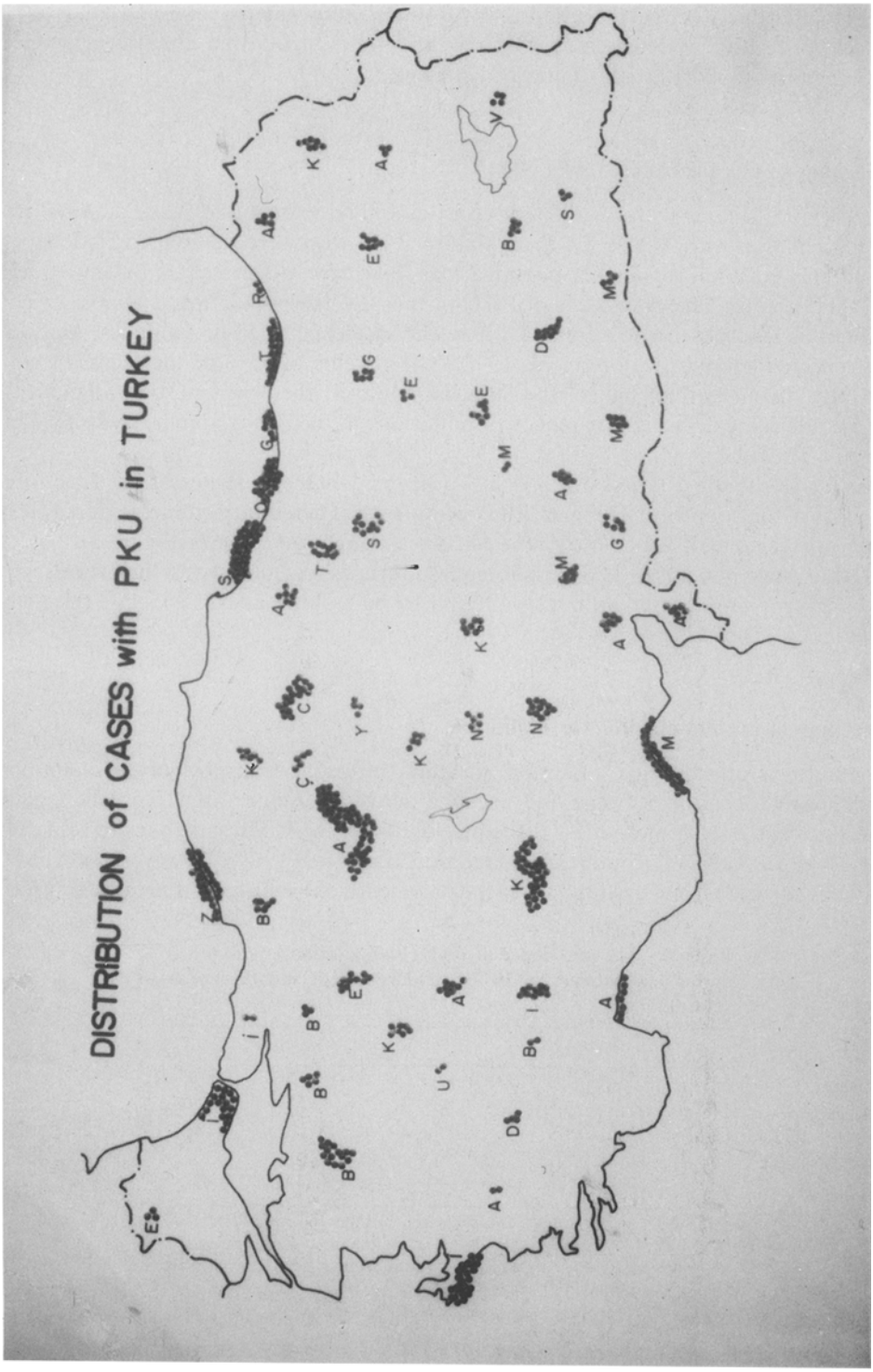

Figure 1 The cases are relevant in all parts of the country. The larger number of dots in certain regions of the country refelected a biased referral pattern.

We have therefore screened 8572 normal elementary school students for cystinuria, and sodium nitroprusside tests were positive in 7 cases. Urinary cystine excretion was $> 300\,mg\,day^{-1}$ and massive increases in lysine, ornithine and arginine were observed in two-dimensional paper chromatography. Although the population screened is small, it seems that the incidence of cystinuria is high in Turkey compared with other countries (Bickel *et al.*, 1975). The causes of urolithiasis, however, are heterogeneous so there is a need to screen a group of patients with urolithiasis to find out the role of cystinuria in urinary tract calculi formation.

Screening results in normal newborns for PKU

A total of 170 000 newborns were screened by the Guthrie test; these infants were born in maternity hospitals in the metropolitan districts of Ankara, Istanbul, Izmir, Samsun, Trabzon and Diyarbakir. Fifty-eight cases with persistent hyperphenylalaninaemia (HPA) were detected (1 in 2785). In 39 of these cases, confirmatory tests were found to be compatible with PKU (1 in 4370) (Table 3). Serum phenylalanine levels have been found to be persistently $8–15\,mg\,dl^{-1}$ on a normal dietary intake in the other 19 cases. Since serum phenylalanine concentrations have never exceeded $10\,mg\,dl^{-1}$ on $2–2.5\,g\,kg^{-1}$ daily protein intake and serum phenylalanine returned to preloading levels within 24 h in 11 of these cases, they were classified as having mild persistent hyperphenylalaninaemia. The PKU/HPA ratio amounted to 2.05 which is comparable to that in other countries (Veale, 1980). Tetrahydrobiopterin (BH_4) loading tests were performed and urinary neopterin and biopterin excretions were determined in 8 out of the 58 infants and found to be not compatible with a defect in BH_4 metabolism. In the other 50 cases, developing normally on a diet restricted in phenylalanine, hyperphenylalaninaemia due to a defect in BH_4 metabolism was not considered.

By taking into account all these results, we would like to conclude that the incidence of many of the inborn errors of metabolism, and specifically the incidence of PKU, is high in Turkey. A population screening programme for PKU in all regions of Turkey should reveal a large number of cases. Since the survey indicates that most of the inborn errors of metabolism result from consanguineous marriage, there is an urgent need to educate the population on the outcomes of such marriages.

Table 3 Incidence of phenylketonuria and hyperphenylalaninaemia among 170 466 newborns screened by the Guthrie test

Type of hyperphenylalaninaemia	*No. of cases detected*	*Incidence*
Typical phenylketonuria	39	1:4370
Persistent hyperphenylalaninaemia	19	1:8971
Total	58	1:2874

Ratio of phenylketonuria/persistent hyperphenylalaninaemia = 2.05

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