Phenylketonuria and glycogen storage disease type III in sibs of one family

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Hyperphenylalaninemia result from a block in the conversion of phenylalanine into tyrosine due to a defect in either the enzyme phenylalanine hydroxylase (98% of subjects) or in the metabolism of the cofactor tetrahydrobiopterin. Phenylalanine hydroxylase deficiency is the most common form of inherited hyperphenylalaninemia disorders, with a prevalence between 1/4,000-1/40,000.

Glycogen storage disease (GSD) type III is caused by debranching enzyme deficiency of glycogen degradation. The clinical features vary in relation to the localization of the enzyme defect. Two clinical entities exist: a combined hepatic myogenic form (GSD IIIa) and a purely hepatic form (GSD IIIb). The inheritance is autosomal recessive.

We describe a Turkish family in which two girls were found to have phenylketonuria, while in two other sisters glycogen storage disease type III was diagnosed. The parents of these children are cousins and they have had 12 children.

Key words: phenylketonuria, debranching enzyme deficiency, consanguinity, glycogen storage disease III.

Hyperphenylalaninemia results from a block in the conversion of phenylalanine into tyrosine due to a defect in either the enzyme phenylalanine hydroxylase (98% of subjects) or in the metabolism of the cofactor tetrahydrobiopterin. Phenylalanine hydroxylase deficiency is the most common form of inherited phenylalanine disorders, with a prevalence of between 1/4,000-1/40,000[1-3]. The gene for hyperphenylalaninemia is mapped to chromosome 12q22; multiple mutations have been identified.

Glycogen storage disease (GSD) type III is caused by a debranching enzyme deficiency of glycogen degradation. The clinical features vary in relation to the tissue localization of the enzyme defect. Two clinical entities exist: a combined hepatic myogenic form (GSD IIIa) and a purely hepatic form (GSD IIIb). The inheritance is autosomal recessive. The gene for the debranching enzyme is mapped to chromosome 1p21[4-6].

We report a Turkish family in which two girls were found to have phenylketonuria (PKU), while in two other sisters GSD type III was diagnosed. The question arises as to whether or not these enzyme defects are associated in some way.

Case Report

We describe a Turkish family in which two girls were found to have phenyketonuria, while in two other sisters GSD type III was diagnosed. The parents of these children are cousins and they had eight more children. Of these, three male children had died: two due to neurologic deterioration and another due to birth trauma. One girl had died as well because of bronchopneumonia complicating measles. They have four healthy daughters who are now married. The family immigrated to the Netherlands 15 years ago.

Patient So. U., nine years old at admission and the ninth child of consanguineous and healthy parents, was admitted for metabolic investigation

because of hepatomegaly, cardiomegaly and attacks of hypoglycemia. Physical examination revealed a girl with thin extremities, height of 126 cm (3rd percentile), weight of 30 kg (weight for height > 90th percentile) and a head circumference of 52 cm (50th percentile). There was a systolic murmur of grade III/VI and the liver was palpable 12 cm below the right costal margin. Laboratory studies showed highly elevated liver enzyme activities (AST, ALT, γ-GT, LDH) and very high CK activity, indicating muscle wasting. Electron microscopy of a liver biopsy revealed massive amounts of intrahepatocytic glycogen.

She is now 22 years old. Physical examination is normal except for two-year history of supraventricular tachycardia, but we consider this problem to be independent of GSD. Abdominal USG and cardiac ECHO are normal. Her psychomotor development appears to be normal. She has been married for two years. She suffers from hypoglycemic symptoms, and thus has to eat every 2-3 hours.

Patient P. U., eight years old on admission and tenth child of the same family, was admitted for the same reasons as her one year older sister. She also had hepatomegaly, cardiomegaly and attacks of hypoglycemia. On physical examination thin extremities were also seen, and a systolic murmur noted. Her height was 116 cm (3rd percentile), weight was 25.5 kg (weight for height > 90th percentile) and she had a head circumference of 51 cm (50th percentile). The liver enzyme activities were highly elevated and CK activity was also high. As in her sister, glycogen storage was confirmed in a liver biopsy.

She is now 21 years old. Physical examination and biochemical findings are normal. There is no hepatomegaly or other pathological findings. She has been married for two years. In both sisters, GSD was diagnosed in Turkey. Type identification was performed in the Netherlands.

In both patients, glucose and fasting glucagon tests were performed. The glucose curves after glucose loading were clearly abnormal in both patients. Zero time values were below normal and the high, biphasic, prolonged curves were suggestive of a glycogen storage disease.

The lactate curves after glucose laoding pointed towards GSD III or VI. The fasting glucagon test excluded phosphorylase b kinase deficiency. The postprandial glucagon test showed an almost flat curve, so we were unable to discriminate between a debranching enzyme or phosphorylase deficiency.

The enzyme determinations in leukocytes, muscle, liver and fibroblasts of the two patients revealed a deficiency of a debranching enzyme consistent with GSD III (Table I), while phosphorylase activity was found to be normal in their erythrocytes (Table II).

Table I. Debranching Enzyme Levels

	Debranching enzyme			
	(nmol/min/mg)			nmol/hr/mg protein
	Leukocytes	Muscle	Liver	Fibroblasts
So.U.	0.4	0.16	ND	1.1
P.U.	0.4	0.13	0.08	1.6
Si.U.	–	–	–	12
Control (s)	1.7	1.76	2.7	13.-40

ND: not determined.

Table II. Phosphorylase Enzyme Levels in Erythrocytes

	Phosphorylase in erythrocytes		
	(nmol/min/mg protein)		(μmol/min/g Hb)
	A	a+b	b kinase
So.U.	0.018	0.036	0.95
P.U.	0.022	0.033	1.1
Control (s)	0.018-0.066	0.035-0.117	0.36-2.78

The enzyme assays in leukocytes, erythrocytes, muscle and liver were performed by Dr. R. Berger, Academic Hospital Groningen and in fibroblasts by Dr. O. P. van Diggelen, Erasmus University, Rotterdam.

Patient E.U., a girl aged four years, was found to have PKU by metabolic investigation after PKU was established in her sister. The girl was admitted to our hospital at the age of five years. Her weight was 18.5 kg (90th percentile), height 101 cm (< 3rd percentile), and head circumference 50.5 cm (50th percentile). She appeared to have psychomotor retardation. She is now 19 years old. She is able to do daily activity and to speak some sentences. She is going to a special school for the mentaly retarded.

Patient Si.U., aged 10 months, was admitted to our hospital for the differential diagnosis of PKU, which was found shortly after birth by routine neonatal screening in Turkey. She had been treated ever since with a phenylalanine restricted diet. On admission she weighed 12 kg (> 95th percentile), her length was 71 cm (10th percentile), and head circumference 46 cm

(75th percentile). Physical examination was normal. She is now 15 years old and a second-year student at college. She has normal mental and neuromotor development.

BH_4 loading test was performed in both patients on admission, and plasma phenylalanine levels did not decrease; six hours after loading the concentrations were similar to starting levels. The concentration of tyrosine did not increase in patient Si.U. and increased only slightly in patient E.U. (Tables III, IV, V, VI).

The biochemical findings indicated PKU caused by liver phenylalanine-hydroxylase deficiency in both patients.

Discussion

Turkey has a high rate of consanguineous marriages (21.1%). Social and cultural factors are especially important in marriages between first and second cousins[7]. Thus, a high prevalence of inherited metabolic diseases is present in Turkey[8]. Despite the lack of offical data, we also assume a high consanguinity ratio in the Turkish population living in the Netherlands. The parents of our patients are first cousins and they have been living in the Netherlands for 15 years.

The clinical manifestations of glycogenosis III tend to be milder than those of type I, but the diseases cannot reliably be distinguished without laboratory procedures. The most consistent clinical feature is an enormously enlarged liver, and it may be the only clinical abnormality at the time of presentation. Hypoglycemia is usually not a prominent feature of this disease, but fasting concentrations of glucose are usually moderately reduced and some patients, especially in infancy, have severe hypoglycemia and even convulsions[4-6]. Our patients had hepatomegaly and hypoglycemic

Table III. Phenylalanine and Tyrosine Concentrations in Plasma and Urine on Admission (in PKU Sibs)

	Plasma (μmol/L)		Urine (μmol/g creatinine)	
	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine
Si.U.	1442	36	3461	236
E.U.	1345	34	1021	83

Table IV. Abnormal Phenylalanine Metabolites in Urine on Admission (in PKU Sibs)

	(μmol/g creatinine)			
	Phenyl-pyruvate	phenyl-lactate	phenyl-acetate	O-OH-phenyl-acetate
Si.U.	1160	1967	2360	460
E.U.	4685	5149	1531	220

Table V. BH_4 Loading Test

		Si.U.		E.U.	
Sampling material	Sampling period (in hours)	Phenylalanine	Tyrosine	Phenylalanine	Tyrosine
Urine (μmol/g creatinine)	12-0 before BH_4	5332	221	–	–
	0-4 after BH_4	5944	435	1827	101
	4-8 after BH_4	6117	387	2344	158
Plasma (μmol/L)	0	2131	87	1440	54
	2 after BH_4	2282	114	1431	45
	4 after BH_4	2238	111	1548	92
	8 after BH_4	2144	91	1571	76

Table VI. Urinary Pterins* (mmol/mol. creatinine) Before BH_4 Loading

	Si.U.	E.U.
Neopterin	4.4	3.2
Monapterin	0.3	0.3
Biopterin	4.5	10.4
Pterin	1.5	0.9
Mol % biopterin	49	75

* This assay was performed by Dr. M. Duran, University Children's Hospital, Het Wilhelmina Kinderziekenhuis, Utrecht.

attacks and patient So.U. also had convulsions. On the other hand, both sisters have normal mental and neuromotor development.

A variety of functional studies, especially glucose tolerance and postprandial glucagon tests, have been employed to document the presence of type III glycogenosis and to distinguish it from types I and VI[9]. However, in both sibs the two-hour postprandial glucagon test gave only a slight glucose response, which is difficult to interpret. In patients with a debranching enzyme deficiency, a higher response is expected. However, another possibility might be a shortage of glycogen containing enough outer branches for conversion to glucose by phosphorylase. Nevertheless, despite these results, the results of other tests were consistent with debranching enzyme deficiency.

The diagnosis of GSD type III could be established by determination of the debranching enzyme in our patients' cells and tissues[10].

At present only limited information is available about the long-term outcome of patients with GSD type III. In one study on 50 patients, hepatomegaly was found to be the most prominent sign in 68% of cases, followed by myopathy in 63%, cardiomyopathy in 50% and hypoglycemia in 8%. Mental development was reported to be normal in 93% of the patients[11]. In our patients, physical and routine laboratory examinations were normal, although patient So.U. suffered from intermittent hypoglycemic symptoms.

Analysis of urine and plasma samples from the GSD III patients for PKU showed no abnormalities. The PKU sibs were not extensively investigated for GSD as they were clinically nonsuspect for this disease. However, debranching enzyme activity in the fibroblasts from Si.U. showed a borderline value (Table I).

Phenylketonuria, an autosomal recessive disorder, occurs in one in 15,000 births and is most common among persons of Western European background. It is probably best characterized by a ratio of blood phenylalanine to tyrosine persistently greater than 3. Phenylketonuria results from a block in the conversion of phenylalanine into tyrosine due to a defect in either the enzyme phenylalanine hydroxylase (98% of subjects) or in the metabolism of the cofactor tetrahydrobiopterin.

The most important and sometimes the only manifestation of PKU is mental retardation. Behavioral difficulties, seizures, rashes, and an unusual body odor may occur[1-3]. Patients with PKU are fair haired, fair skinned and have blue eyes in over 90% of the untreated or later-treated cases, as was seen in the late-treated patient E.U.[12].

All subjects with hyperphenylalaninemia should be screened for disorders of biopterin metabolism[2]. After BH_4 loading test, our patients' plasma phenylalanine levels did not decrease; six hours after loading the concentrations were similar to starting levels. The concentration of tyrosine did not increase in patient Si.U., and only slightly increased in patient E.U. BH_4 loading test results in both patients were consistent with classic PKU.

In Turkey, PKU neonatal screening started 15 years ago. Patient Si.U. was diagnosed after the first neonatal screening in Turkey. The different clinical course seen in patients Si.U. and E.U. dramatically highlights the importance of PKU screening.

The incidence of PKU in Turkey is the highest recorded in any country (1:6,000)[13]. GSD is also frequently seen in Turkey due to the high consanguinity rate, but the distribution of various types of GSD is unknown[14]. The overall frequency of all forms of GSD based on European data is approximately 1 in 20,000 to 25,000 live births[4]. Because the incidence of GSD is also estimated to be high in Turkey, the existence of both diseases in one family could have been coincidental.

Association of PKU with scleroderma, Duchenne muscular dystrophy, Charcot-Marie-Tooth disease, Down syndrome, cystinuria, homozygous hypobetalipoproteinemia, and bilateral iris coloboma and optic atrophy has been reported previously[15]. Coşkun et al.[15] also described a three-year-old Turkish girl with PKU and hereditary fructose intolerance. As far as we know the combination of PKU and GSD III in one sibship has not been reported in the literature. The clinical history was reported without typing the GSD[16], thus this is the first full report on the combination of PKU and GSD in one sibship.

Every person is a carrier of at least six-to autosomal recessive disorders. Therefore the chance of having two inborn errors in the offspring of a consanguineous couple is not negligible. It is in this light even suprising that so few "double inborn errors" occur in highly in bred populations.

REFERENCES

1. Scriver CR, Kaufman S, Eisensmith RC, Woo SL. The hyperphenylalaninemias. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Disease (7th ed) Vol. I. New York: McGraw Hill, Inc.; 1995: 1015-1075.
2. Smith I, Lee P. Hyperphenylalaninemias. In: Fernandes J, Saudubray JM, van den Berghe G. (eds). Inborn Metabolic Disease (3rd ed). Berlin, Heidelberg, New York: Springer-Verlag, 2000: 170-184.
3. Wappner R, Cho S, Kraonmal R, Schuett V, Seashore MR. Management of phenylketonuria for optimal outcome: a review of guidelines for phenylketonuria management and report of surveys of parents, patients, and clinic directors. Pediatrics 1999; 104: 68.
4. Chen YT, Bruchel A. Glycogen storage disease. In: Scriver CR, Beaudet AL, Sly WS, Valle D (eds). The Metabolic and Molecular Bases of Inherited Disease (7th ed) Vol. I. New York: McGraw Hill, Inc.; 1995: 935-966.
5. Nyhan WL, Ozand PT. Glycogenosis type III. In: Nyhan WL, Ozand PT (eds). Atlas of Metabolic Disease. London: Chapman Hall Medical; 1998: 348-355.
6. Fernandes J, Smit GP. The glycogen storage diseases. In: Fernandes J, Saudubray JM, van den Berghe G. (eds). Inborn Metabolic Disease (3rd ed). Berlin, Heidelberg, New York: Springer-Verlag; 2000: 86-101.
7. Tunçbilek E, Koç I. Consanguineous marriage in Turkey and its impact on fertility and mortality. Ann Hum Genet 1994; 58: 321-329.
8. Özalp I, Coşkun T, Tokol S, Demircin G, Monch E. Inherited metabolic disorders in Turkey. J Inherit Metab Dis 1990; 13: 732-738.
9. Perkoff GT, Parker VJ, Hahan RE. The effects of glucagon in three forms of glycogen storage disease. J Clin Invest 1962; 41: 1099.
10. Shin YS. Diagnosis of glycogen storage diseases. J Inherit Metab Dis 1990; 13: 419-434.
11. Smit GP, Fernandes J, Leonard JV, et al. The long term outcome of patients with glycogen storage disease. J Inherit Metab Dis 1990; 13: 411-418.
12. Kaufman S, Holtman NA, Milstien S, Butler LJ, Krumholz A. Phenylketonuria due to deficiency of dihydropteridine reductase. N Engl J Med 1975; 293: 785-790.
13. Özalp I, Coşkun T, Tokatlı A, et al. Neonatal PKU screening in Turkey: 7 years experience in a developing country. Screening 1995; 4: 139-147.
14. Saltık IN, Özen H, Ciliv G, et al. Glycogen storage diseases type Ia: frequency and clinical course in Turkish children. Indian J Pediatr 2000; 67: 497-501.
15. Coşkun T, Özalp I, Tekinalp G. Hereditary fructose intolerance in a patient with phenylketonuria. Turk J Pediatr 1991; 33: 181-184.
16. Coşkun T, Özalp O, Koçak N, Tekinalp G. Aynı ailede fenilketonuri ve glikojenozis. Çocuk Sağlığı ve Hastalıkları Dergisi 1986; 29: 233-237.