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Phenylketonuric patients decades after diet

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Summary: Nineteen early-treated phenylketonuric patients, whose diet was discontinued between 4.5 and 13 years of age, and who have been off the diet for 12–28 years, were reassessed in 1992–93. There was little change in mean IQ between end of diet and follow-up, less than one IQ point on the average, with no change for any individual exceeding 12 IQ points. Both prior and current IQ correlated slightly negatively with mean phenylalanine (Phe) concentration, and positively with parents' education. The phenylalanine level at follow-up was significantly lower on average by about 900 μmol/L. Five of the subjects (26%) have evidence of mental disease. However, the data suggest that the discontinuation of the diet did not cause intellectual deterioration. Nonetheless, the patients' intellect cannot be the only consideration for maintenance of diet. The occurrence of psychopathology among phenylketonuric patients and the possible unknown effects of toxic elevation of phenylalanine during their lifetime suggest the need to maintain the diet. The use of DNA for diagnostic and prognostic purposes might assist in decisions about dietary quality and duration, and in anticipation of psychopathology.

Before 1980, in most treatment centres, the diet for treatment of phenylketonuria (PKU) was discontinued prior to school years (Schuett et al 1980). Some reports showed no intellectual deterioration following discontinuation of the diet (Kang et al 1970; Holtzman et al 1975; Koch et al 1982; Chang et al 1983; Saudubray et al 1987). Currently the majority of centres treating PKU recommend indefinite continuation of the diet (Schuett and Brown 1984), in part because other publications suggest a decrease of intellect in those whose diet was stopped early (Smith et al 1978; Koch et al 1982; Seashore et al 1985; Matthews et al 1986; Fishler et al 1989). This is caused partly by the fact that PKU is a metabolic disease and the 'damaging effect' might manifest itself not only in changes in intellectual performance but also in school or other kinds of performance. Nevertheless, dietary control becomes increasingly difficult with advancing age, and the fear of possible

deterioration, without substantial evidence, cannot be sufficient to recommend maintaining the diet.

We present here the status of 19 PKU adults whose diet was discontinued many years ago. The time between the discontinuation of diet and the follow-up is the longest so far reported.

METHODS

Forty-two PKU patients, who started the diet within 2 months of birth and discontinued some years later, form the study population. Eighteen patients could not be traced. The remaining 24 patients were asked to return to the clinic; five refused. The study for the remaining 19 PKU patients was started in February 1992 and completed by May 1993. Eighteen of the 19 patients were found by routine newborn screening. The nineteenth (the oldest one) was tested after birth because the child had known retarded siblings. During dietary treatment of the patients, serum phenylalanine was measured by fluorimetric methods (McCaman and Robins 1962). Each patient had blood taken for phenylalanine and tyrosine determination by high-performance liquid chromatography. Mutation analysis was performed in 17 patients according to previously described procedures (Guldberg et al 1993a,b). Briefly, genomic DNA was isolated from peripheral blood leukocytes. The entire coding sequence and all intron/exon boundaries of the PAH gene were scanned for mutations by polymerase chain reaction (PCR) in combination with denaturing gradient gel electrophoresis (DGGE). Fragments containing a sequence variant as determined by DGGE were subjected to direct sequence analysis for further mutation identification. All subjects participated in a structured interview and were given an individual intelligence test, the Wechsler Adult Intelligence Scale-Revised. Each patient had yearly development tests from 6 months of age. For this study we used their last IQ test (WISC) prior to discontinuation of the diet and compared it with the current one. The structured interview was designed for this study, specifically to obtain data about each subject's educational level, working status, marital or dating status, cohabitation status, whether the patient had children or intended to have children in the future, and whether the individual had a history of mental illness diagnosed by a psychiatrist or a psychologist. The data are presented as they were at the time of the patient's visit.

RESULTS

Table 1 summarizes some measurements on these patients. The proportion of females was 10/19; 6/19 have been married, and 4/19 have had children. On average, the patients started their diet within the first month of life, and all had started by age 54 days. They remained on the diet between 33 and 132 months (mean 77 months, or about 6.5 years). The patients have now been off diet for at least 12 years.

Phenylalanine concentration: Three measurements of phenylalanine concentration are included in this study: the initial reading at diagnosis; the average reading while diet was being followed; and a measurement taken at the follow-up screening. The initial Phe concentrations ranged between 1090 μmol/L and 4358 μmol/L, with a mean of 2450 μmol/L. While on the diet, Phe concentrations were controlled to a more acceptable

Table 1 Univariate summary statistics

Measurement	*Mean*	*SD*	*Minimum*	*Maximum*
Diet age (age in days at start of diet)	25.1	15.7	10	54
Diet length (months on diet)	76.7	20.6	33	132
Years off diet (years off diet until follow-up)	19.1	4	12	28
Initial Phe concentration (μmol/L)	2450	955	1090	4358
Mean diet Phe concentration (μmol/L)	683	188	351	999
Follow-up Phe concentration (μmol/L)	1542	381	908	2300
Last IQ when on diet	92.8	18.8	64	123
IQ now (follow-up IQ)	93.0	15.8	72	119
Years of education	13.7	2.2	11	18
Parents' average years of education	13.6	3.3	9.5	20
Age diet stopped (months)	78.5	17.9	57	132
Age at follow-up (years)	24.5	4.3	18	33

351–999 μmol/L, with a mean value of 683 μmol/L. At follow-up, concentrations had increased to 908–2300 μmol/L, much higher than those on diet but still considerably lower than the values at diagnosis.

IQ Scores: The data indicate remarkably little change in IQ since the end of the diet, with change in mean between the end of diet and follow-up of less than one IQ point (paired $t=0.13$, df=18, $p>0.5$). No change for any individual exceeded 12 IQ points. The correlation between the two IQ measurements is 0.84. No relationship is apparent between follow-up Phe concentration and follow-up IQ level ($r=0.08$, $p>0.5$).

Mental illness: Five of the 19 patients were diagnosed with some form of mental illness at follow-up: two with depression, one with impulse control disorder, one with simple phobia, and one with dysthymia. Occurrence of mental illness was more frequent in patients with lower IQ or lower education. Mean years of education for patients with mental illness was 11.8 years; the mean for the remaining patients was 14.4 years ($F=6.82$, $p>0.02$). For follow-up IQ, mean difference was 23.3 points in favour of the group with no mental illness ($p<0.01$). No differences ($p>0.3$) in Phe levels for the two types of patients were observed. The other 14 patients did not have psychiatric evaluations or reported problems.

Parents' education: Parents' average education is about the same as the patient's education. Parents' education is slightly predictive of the patient's IQ at follow-up ($r^2=0.26$, $p<0.03$) but is otherwise unrelated to the other measurements on these patients.

DNA analysis: DNA analysis was completed on 17 of the 19 patients (Table 2). Of these, 3/17 (18%) had a mutation with potential influence on enzyme activity identified on only one of two alleles; the remaining 14/17 (82%) had a mutation present on both alleles. Of these 14, 4/14 (29%) had inherited on one chromosome a mutation associated with mild PKU (nos 10, 11, 18 and 19).

The patients can be divided into three groups: 3 patients not completely characterized

Table 2 University of Minnesota PKU follow-up study

ID no.	*Prior diet Phe*[a]	*Follow-up Phe*[b]	*Diet IQ*[c]	*IQ now*[d]	*Mental illness*	*Allele 1*	*Allele 2*
1	2603	1513	107	96	No	R408W	R252Q
2	2845	1695	102	109	No	IVS-12nt1	Y356X
3	2361	2058	113	102	No	IVS-12nt1	Y356X
4	3329	1392	99	93	No	R408W	IVS-12nt1
5	4358	1816	64	73	No	R408W	nd[e]
6	3935	1937	66	74	No	R408W	nd[e]
7	1816	1816	123	111	No	R408W	R243X
8	2906	1816	74	77	Yes	R408W	IVS-12nt1
9	3935	1271	71	75	Yes	R408W	R158Q
10	1816	1513	97	98	No	R252W	DI94
11	1150	908	101	95	No	R408W	Y414C
12	2119	1029	94	97	No	–	–
13	2663	1755	120	119	No	R408W	L348V
14	1574	1634	113	118	No	nd[e]	Y386C
15	2421	1150	78	80	Yes	R408W	IVS-12nt1
16	1574	2300	86	95	No	R408W	IVS-12nt1
17	1090	1332	72	75	Yes	–	–
18	1392	969	106	108	No	P281L	F39L
19	2663	1392	77	72	Yes	R408W	A104D

[a]Prior diet Phe = phenylalanine concentration before diet (μmol/L)
[b]Follow-up Phe = follow-up phenylalanine concentration during dietary treatment (μmol/L)
[c]Diet IQ = Last IQ score before going off diet
[d]IQ now = IQ score at follow-up
[e]nd = not determined

with respect to mutation genotype; 4 patients associated with mild PKU; and 10 patients completely characterized with respect to mutation genotype. At all phenylalanine concentrations, the patients not completely characterized with respect to mutation genotype have the highest mean phenylalanine, although only at the most recent measurement is the difference close to significant (group not completely characterized, mean 1796 μmol/L, $p < 0.04$). No differences in IQ ($p < 0.5$) are apparent for the three groups.

DISCUSSION

The PKU patients presented here were born between 1960 and 1973. Their diet was discontinued primarily because at that time it was generally accepted practice to discontinue diet prior to entering school, partly because of difficulties in maintaining the diet. The patient kept on diet longest had two untreated retarded PKU siblings. The dietary treatment for PKU has been modified considerably since then. The intellectual outcome of patients depends on good control during the first 8–10 years of age (Holtzman et al 1986; Michals et al 1988; Smith et al 1991), but there is an association of decline in intelligence with higher phenylalanine concentration after 8 years of age (Smith et al 1991). The patients reported here were among the earliest treated; therefore, the intellectual outcome of the PKU patients in this report is not as good as that of patients treated currently, but the results are compatible with results for others of that period (e.g. mean IQs of 94

reported) (Dobson et al 1976). Children born in the 1960s did worse than children born in the 1970s (Smith et al 1991). Six of the 19 patients' IQs are less than 80, and four of these are siblings (nos 5, 6, 9 and 17). For these six, mean phenylalanine levels during the diet were usually in the higher range and their parents' education was less than that of others. Although the mean IQs of the studied patients were lower than in recent reports, the negative influence of the quality of the diet measured by higher phenylalanine, as well as the positive effect of parents' higher education, was similarly observed (Saudubray et al 1987).

Attempts to randomize early-treated PKU children failed to keep them in two groups (Koch et al 1985). Similarly, randomization has failed to give a single longitudinal prospective study to assess the effects of discontinuation of diet prior to adolescent years. Such a study can no longer be expected to be done in our ethically and legally volatile environment. In a randomized control study of PKU patients whose diet was discontinued at 4 years of age, no harmful effects of diet termination were noted, but a longer period of observation was suggested (Holtzman et al 1975).

One cannot assume that early dietary treatment itself is the only solution for the handicaps of PKU patients, nor can IQ scores be the sole measure of their well-being. PKU plus diet does not equal normal (see Chang et al 1983). Children with early-treated PKU were selectively impaired in executive function (maintenance, planning, organization) measures, even when still on the diet; however, the executive function score was negatively related to phenylalanine (Welsh et al 1990). Additional neuropsychopathological manifestations of PKU confront clinicians. Concern about school performance, with or without intellectual deterioration, is also increasing. However, mean scores of arithmetic, language and perceptual skill declined at a uniform rate in those who were treated and those whose diet was discontinued (Fishler et al 1989). Decline of social skills was reported following dietary discontinuation after 5.5 years of age (Matthews et al 1986). Some isolated reports described neurological deterioration after discontinuation of the diet (Wood 1976; Thompson et al 1990; McCombe et al 1992). Changes following discontinuation of the diet in adolescents and young adults for neurotransmitter metabolism are reported (Lou et al 1985). MRI findings following dietary changes reflect biochemical control, rather than indicating significant neurological damage (Cleary et al 1994).

Early-treated adolescent patients saw their whole social situation as distinctly restricted; the majority had great difficulty managing their diet, and for up to 15 years their phenylalanine levels were persistently above the desired range (Weglage et al 1992). There were difficulties in maintaining the diet at a later age, and phenylalanine levels of 900–1100 μmol/L (15–18 mg/dl) did not have a beneficial effect on difficulties in school performance (Michals et al 1985).

The occurrence of psychopathology among PKU patients is alarming, and might be a complication of older PKU patients, after elimination of retardation in young PKU patients. These data also show a high incidence of psychopathology. This might mean that discontinuation of the diet enhanced the manifestation of that pathology or that, just as in the general non-PKU population, it was manifested at a more advanced age. Whether or not dietary restriction influences psychopathology remains to be seen.

It would be an oversimplification to expect that diet alone will determine the outcome

of PKU (Chang et al 1983). It is obvious that parents of higher intelligence not only have potentially brighter children but also are more understanding of and comply better with the restrictions of the diet. It is not uniformly accepted that the diet is needed for a lifetime, and studies also suggest that, after 10 years of age, intellectual deterioration due to high phenylalanine concentrations appears to diminish (Smith et al 1991). Intellectual performance seems to be stable after 10 years of age. Although the relationship between better dietary control and intellectual achievement is generally accepted at an early age, not only does dietary restriction become difficult but also its importance is questioned at a later age. Nevertheless, even at a very late age, profoundly retarded PKU patients might show considerable personality improvement (again not uniformly) soon after the diet is instituted.

This conflicting information puts clinicians in a very difficult situation, not only about whether the diet should or should not be maintained but also in justifying the intention to maintain a very restricted diet at a later age. The advances in DNA technology might be able to clarify some of the issues, such as the severity of PKU, the need for restriction, duration or expected psychopathology. The clinical variations and the severity of PKU alleles are determined by genotype. The spectrum of phenylalanine hydroxylase activities from 0 to 50% of normal activity depends on the mutant allele present (Okano et al 1991). It is now possible to predict the metabolic phenotype of a hyperphenylalaninaemic neonate, thereby improving the design of optimal dietary therapy (Güttler et al 1992).

PKU is a genetically determined enzyme deficiency and, as long as genetic engineering is not available, the possible consequences of abnormal metabolism have to be considered for a lifetime. However, until gene therapy is available, new methodology might better assist clinicians in managing PKU; until then, the generalized statement regarding the extension of diet through adulthood for fear of loss of intellect for PKU patients is scientifically unfounded.

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