# Detection of Phenylketonuria Carriers

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THE ANALYSIS of single blood samples for phenylalanine (PAA) and tyrosine (*1*) simplifies the identification of phenylketonuria (PKU) carriers and may replace the tolerance test, with its multiple samples and occasional sympathomimetic responses (*2*). The new method's potential for wide application prompted us to survey several families in New York State's PKU register (*3*). Our results corroborate Canadian reports of its validity in presumed carriers (*1,4*) and illustrate its adaptability to the screening of high-risk groups.

## Methods

From 59 members of nine families on the PKU register, heparinized blood samples (375 microliters) were collected in their homes by finger puncture after the persons had fasted overnight. These 59 relatives of persons with phenylketonuria consisted of 17 parents (referred to hereafter as the presumed carriers), two great grandparents, 12 grandparents, six siblings, 17 aunts and uncles, and five cousins. In two of the 11 probands, M. M. and H., the defect had been detected in infancy through New York State's mandatory PKU screening program; other probands resided at, or were outpatients of, the New York State Department of Mental Hygiene's schools for the mentally retarded. Similar blood samples from 13 healthy young adults with no family history of phenylketonuria served as controls. Twenty-seven of the relatives were male, as were eight of the controls.

The blood samples were delivered or mailed to the PKU screening laboratory of the division of laboratories and research, State of New York Department of Health, within 24 hours and were deproteinized before assay (*5*). Phenylalanine concentrations were estimated by the spectrophotofluorometric method described by McCaman and Robins (*6*); tyrosine concentrations by the method Udenfriend (*7*) and J. Bourdillon, senior medical biochemist in the division of laboratories and research, have described. The standard deviation in 136 assays of the Hyland standard with a mean PAA concentration of 2.47 mg. per 100 ml. of blood was $\pm 0.30$; the deviation in 20 assays of the Dade standard (Monitrol 1), with a mean tyrosine concentration of 1.43 mg. per 100 cc., was $\pm 0.21$. Variation in the concentrations of the two amino acids in samples from the same person obtained on different days was within 10 percent.

We assessed the effect of mailing on samples by comparing the PAA and tyrosine concentrations in several which were assayed on the day of sampling and in aliquots of the same samples assayed after 24 hours in transit. Concentrations of both of the amino acids were greater in the

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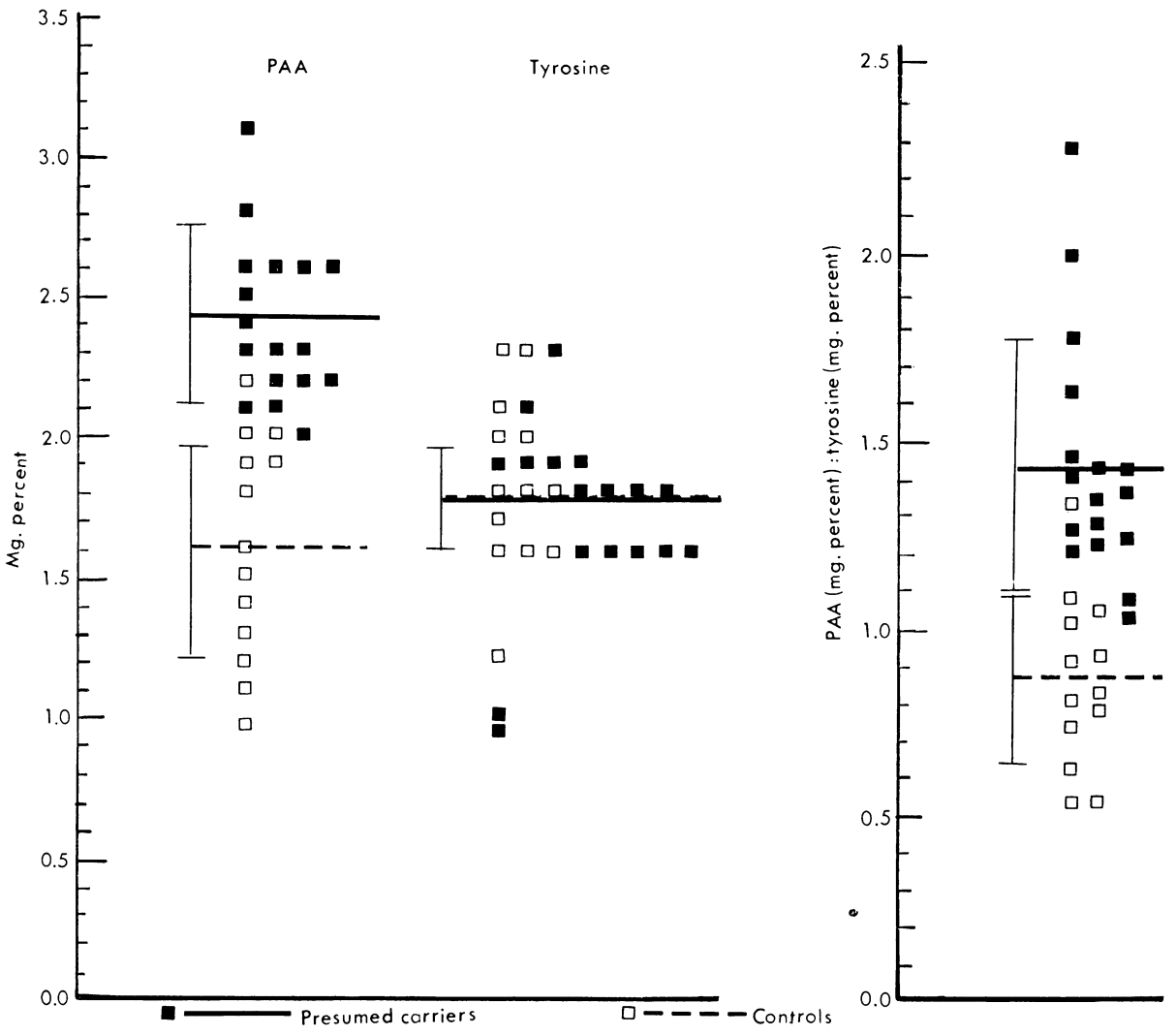
mailed samples, but as other investigators have previously noted (*8*), the increases were within the limits of the assays' precision.

## Results

The figure illustrates the means, standard deviations (S.D.), and ranges of the fasting plasma PAA and tyrosine concentrations and of the PAA to tyrosine ratios found in the 17 presumed carriers and the 13 controls. PAA concentrations in all but one of the control samples fell within or below the 95 percent confidence limits of the mean of 1.6 mg. per 100 ml. of blood, S.D. ±0.37; all but one of the samples from the presumed carriers were within or above the confidence limits of the mean of 2.4 mg. per 100 ml., S.D. ±0.32. Phenylalanine concentrations were the same (2 mg. per 100 ml.) in samples from two controls and one presumed carrier.

Mean concentrations of tyrosine were similar in the carriers and the controls, as others have reported (*1*, *4*). The tyrosine concentration of the one control sample with excessive PAA was well below the 95 percent confidence limits of the mean of 1.8 mg. per 100 ml.; the two samples with abnormal values may have come from heterozygotes.

**Distribution of phenylalanine and tyrosine and of phenylalanine to tyrosine ratios among 17 presumed carriers of phenylketonuria and 13 normal adults**



The ratios of PAA to tyrosine in all but one of the control samples fell within or below the 95 percent confidence limits of the mean ratio of 0.90, S.D. ±0.22; the ratios in the samples from the presumed carriers were within or above the confidence limits of the mean ratio of 1.40, S.D. ±0.37. The ratios for three of the control samples and for two of the samples from presumed carriers overlapped in the range 1.04 to 1.08. These differences in concentrations and the overlapping values are similar to ones mentioned in initial reports of the method (*1*, *4*).

The controls and presumed carriers in our population were as well distinguished by their fasting PAA concentrations as by their ratios of PAA to tyrosine. Since, however, in previous applications of the method, inclusion of the PAA to tyrosine criterion had reportedly reduced the zone of overlap between controls and

**Table 1. Concentrations of phenylalanine, phenylalanine to tyrosine ratios, and phenotype for 59 relatives of the 9 proband families**

| Probands | Great grandparents | | | Grandparents | | | Parents | | | Siblings | | | Aunts and uncles | | | Cousins | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P | R | S | P | R | S | P | R | S | P | R | S | P | R | S | P | R | S |
| N. B | | | | | | | 2.2 | 1.44 | C | 2.0 | 1.40 | C | 3.0 | 1.35 | C | | | |
| | | | | | | | 2.2 | 1.44 | C | 2.6 | 1.24 | C | 2.0 | 0.97 | NC | | | |
| | | | | | | | | | | 2.5 | 1.29 | C | | | | | | |
| H | 2.4 | 1.03 | I | 1.4 | 0.87 | NC | 2.6 | 1.26 | C | 2.3 | 1.24 | C | 3.1 | 1.37 | C | 2.7 | 1.37 | C |
| | 2.3 | 1.00 | I | 1.6 | 0.97 | NC | 2.3 | 1.24 | C | | | | 2.0 | 1.01 | NC | 3.8 | 1.74 | C |
| | | | | 2.9 | 0.87 | I | | | | | | | 2.6 | 1.55 | C | 4.2 | 1.45 | C |
| | | | | | | | | | | | | | 2.0 | 1.03 | NC | | | |
| | | | | | | | | | | | | | 2.2 | 0.84 | I | | | |
| R. T | | | | | | | 2.2 | 2.04 | C | | | | | | | | | |
| | | | | | | | 2.3 | 1.48 | C | | | | | | | | | |
| S. E | | | | 2.9 | 1.47 | C | 2.8 | 1.79 | C | 2.9 | 1.51 | C | 2.3 | 1.41 | C | | | |
| | | | | | | | 2.6 | 1.35 | C | | | | 1.9 | 1.02 | NC | | | |
| | | | | | | | | | | | | | 2.4 | 1.55 | C | | | |
| | | | | | | | | | | | | | 2.3 | 1.18 | C | | | |
| M. M | | | | 2.2 | 1.43 | C | 3.1 | 1.36 | C | | | | 2.5 | 1.29 | C | | | |
| | | | | 2.2 | 0.92 | I | 2.5 | 1.29 | C | | | | 1.8 | 0.99 | NC | | | |
| | | | | 1.5 | 1.30 | I | | | | | | | 2.4 | 1.43 | C | | | |
| | | | | 2.2 | 1.04 | C | | | | | | | | | | | | |
| L. F | | | | | | | 2.3 | 1.27 | C | | | | | | | | | |
| | | | | | | | 2.1 | 1.08 | I | | | | | | | | | |
| P. J | | | | 2.4 | 1.01 | I | 2.0 | 1.04 | I | 2.2 | 1.32 | C | 2.3 | 1.48 | C | 2.1 | 1.04 | I |
| | | | | 2.3 | 1.18 | C | | | | | | | | | | 2.0 | 0.85 | NC |
| W | | | | | | | 2.55 | 1.42 | C | | | | | | | | | |
| | | | | | | | 2.05 | 2.27 | C | | | | | | | | | |
| H.M | | • | | 2.9 | 1.24 | C | 2.55 | 1.64 | C | | | | 2.3 | 1.45 | C | | | |
| | | | | 2.3 | 1.02 | I | 2.4 | 1.24 | C | | | | 2.7 | 1.20 | C | | | |

P—concentration of phenylalanine in mg. per 100 ml. of blood, R—ratio of phenylalanine to tyrosine, S—carrier status, C—carrier, NC—noncarrier, I—indeterminate.

presumed carriers (*1*, *4*), we included these ratios in our interpretation of phenotype in the families studied.

Biochemical indices similar to those of the presumed carriers—PAA concentrations of 2.2 mg. per 100 ml. or more and ratios of 1.08 or more—were found in samples from 23 of the other 42 relatives (table 1). Values similar to those of the control group—PAA concentrations of 2.0 mg. or less and ratios of 1.04 or less—were found in nine samples. One or the other of the two indices characteristic of the presumed carriers, but not both, was found in 10 samples. In eight of the 10, PAA concentrations of 2.2 mg. per 100 ml. or more were observed, for one of which there was an "overlapping" ratio of 1.04; the ratios for two samples were 1.08 or more, for one of which an "overlapping" PAA concentration of 2.0 mg. per 100 ml. was noted.

Relatives were considered probable carriers if both the PAA concentration and the PAA to tyrosine ratio were similar to those for the presumed carriers or if one abnormal parameter was accompanied by the other one in the "overlapping" range. Relatives were considered noncarriers if both biochemical indices were similar to those for the control group. The relative's status was considered indeterminate if only one parameter was abnormal or if both parameters were in the "overlapping" range (table 1).

Fifty-five percent (23 of 42) of the relatives had biochemical characteristics like those of the presumed carriers, a proportion comparable to the prediction of 60 percent made from tolerance tests in a similar population (*2*). The distribution and frequency of the carriers identified in our study, with the exception of a surplus among aunts and uncles, approached the pattern and number that would be expected for a disease with an autosomal recessive pattern of inheritance (table 2).

## Discussion

The identification of carriers may be helpful in distinguishing the classic form of phenylketonuria from other forms of hyperphenylalaninemia detected in birth screening programs (*9*, *10*). A convenient one-sample assay should encourage this approach (*11*).

The validity of the assay as a marker of the carrier state has already been explored through parallel assays of presumed carriers by both methods (*1*). In these assays, the fasting PAA to tyrosine ratios proved as discriminatory as the older tolerance tests (*12–17*), distinguishing about 80 percent of the presumed carriers with biochemical evidence of heterozygosity. Thus, the limited expression of phenotype in a few of our presumed carriers that resulted in a narrow zone of overlapping values with the controls is not unusual. The zone, which occupied about 10 percent of the distribution of PAA concentrations and PAA to tyrosine ratios encountered in our samples, was similar to that found in the two populations previously tested by this method (*1*, *4*). The zone of overlap, however, was less than that found when fasting PAA concentrations alone were compared (*18*) and was not much greater than that obtained in a sensitive loading dose test (*2*).

## Summary

The single fasting-sample procedure of Perry for distinguishing carriers of phenylketonuria was applied to a high-risk population in New York State—59 members of nine families in which persons with the disease had been identi-

**Table 2. Frequency of carriers among various relatives of probands**

| Relationship to proband | Theoretical carrier rate | Relatives sampled | Carriers found | Carriers expected |
|---|---|---|---|---|
| Great grandparents | 2 of 8 (1 in 4) | 2 | 0 | <1 |
| Grandparents | 2 of 4 (1 in 2) | 12 | 5 | 6 |
| Parents | 2 of 2 (1 in 1) | 17 | 15 | 17 |
| Siblings | 2 of 3 (2 in 3) | 6 | 6 | 4 |
| Aunts and uncles | 1 of 3 | 17 | 11 | 6 |
| First cousins | 2 of 12 (1 in 6) | 3 | 1 | <1 |
| Second cousins | 4 of 48 (1 in 12) | 2 | 2 | <1 |

fied. More than 50 percent (23 of 42) of the close relatives of the persons with the disease were found to have biochemical indices similar to those of the 17 presumed carriers (the parents of the probands). The method proved as discriminatory as older procedures and, in general, revealed the expected frequencies of phenotypes. Application of the method in birth screening programs and genetic counseling is therefore feasible.

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