Impact of the Phenylalanine Hydroxylase Gene on Maternal Phenylketonuria Outcome

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ABSTRACT. Objective. The aim of the present study was to examine to what extent maternal and offspring phenylalanine hydroxylase (PAH) genotypes in conjunction with maternal IQ and dietary control during pregnancy are related to cognitive development in offspring of women with phenylketonuria (PKU).

Methods. PAH gene mutations were determined in 196 maternal PKU subjects and their offspring. The women were grouped according to PAH genotype, which predicts the metabolic phenotype (severe PKU, mild PKU, and mild hyperphenylalaninemia [MHP]). IQ was determined in both the mothers (Wechsler Adult Intelligence Scale–Revised at >18 years) and their children (Wechsler Intelligence Scale for Children–Revised at ≥6–7 years of age).

Results. According to PAH genotypes, 62% of the women exhibited severe PKU, 19% exhibited mild PKU, and 19% exhibited MHP. Maternal IQ increased, and the assigned phenylalanine (Phe) levels decreased with decreasing severity of PAH genotype. In offspring of mild maternal PKU, multiple regression analysis showed offspring IQ to be significantly related to maternal IQ but not to Phe exposure during pregnancy, which was <750 μmol/L in all cases of mild PKU. In offspring of mothers with severe PKU and average Phe exposure during pregnancy of 360 to 750 μmol/L, multiple regression analysis revealed both maternal IQ and Phe exposure to be significant predictors of offspring IQ. When average Phe exposure was <360 μmol/L, cognitive development was normal (mean IQ: 105), whereas an average Phe exposure of >750 μmol/L severely depressed offspring IQ (mean IQ: 56) in this group regardless of maternal IQ. It could not be documented that the offspring PAH genotype affects cognitive development.

Conclusion. Female individuals with severe PKU should be offered a diet for a lifetime. If good metabolic control is established, then women with PKU will have children with IQ scores that are not influenced by their disease. Pediatrics 2003;112:1530–1533; PKU, phenylalanine hydroxylase, mutation, maternal PKU, cognitive development.

Abbreviations. PAH, phenylalanine hydroxylase; PKU, phenylketonuria; MHP, mild hyperphenylalaninemia; MPKUCS, Maternal PKU Collaborative Study; Phe, phenylalanine; WISC-R, Wechsler Intelligence Scale for Children–Revised; MPKU, maternal phenylketonuria; APL, assigned blood Phe level.

More than 400 different disease-associated phenylalanine hydroxylase (PAH) gene mutations have been reported to the PAH Mutation Analysis Consortium Database (http://www.pahdb.mcgill.ca). These mutations can, in theory, form >80 000 heteroallelic genotypes, ie, genotypes composed of 2 different mutant alleles. In a previous multicenter study,2 the phenotype associations of >100 of these mutations were suggested. Four arbitrary phenotype categories were used: classic phenylketonuria (PKU), moderate PKU, mild PKU, and mild hyperphenylalaninemia (MHP). The present report is based on the concept that the PAH genotype can be used for classification of individuals with PAH deficiency, in that the metabolic phenotype is determined by the milder of the 2 inherited PAH mutations.1,2

In a previous study, we identified 71 different PAH mutations on 294 independent mutant chromosomes of individuals who had PAH deficiency and were living in the United States and were enrolled in the Maternal PKU Collaborative Study (MPKUCS).3 The 3 most common mutations together accounted for 49% of the mutant chromosomes. The classification of PAH deficiency on the basis of PAH genotypes is used in the present study to determine the relationships among genotype, biochemical phenotype, and cognitive performance (Wechsler Adult Intelligence Scale–Revised4) in PKU women and to explore the impact of maternal genotype, maternal IQ, and average phenylalanine (Phe) exposure during pregnancy on the cognitive development (Wechsler Intelligence Scale for Children–Revised5[WISC-R]) of their children. The influence of offspring genotype on cognitive development is also examined.

METHODS

Patients

Blood samples for genotyping on 236 women who had hyperphenylalaninemia and were enrolled in the MPKUCS were contributed by 90 clinics in the 3 countries involved (United States, Canada, and Germany). The data were compiled and prepared for statistical analyses at the Coordinating Center at the Children’s Hospital and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts; §University of Texas Medical Branch, Children’s Hospital, Galveston, Texas; #University of Tübingen, Reutlingen, Germany; and **National Institute of Child Health and Human Development, Bethesda, Maryland.
Hospital, Los Angeles. Informed consent for enrollment in the study was obtained on each enrollee by the coordinator or geneticist who was caring for each pregnancy. Included in this report were 196 women with PKU and their offspring. Maternal genotype, maternal IQ, and offspring IQ were also available.

Materials

The routine MPKUCS protocol has already been described.6-8 The cognitive performances were determined in the women with PKU after 18 years of age by means of the WAIS-R and in the offspring at 6 to 7 years of age or later by means of the WISC-R. Data on the cognitive abilities of the PKU women’s husbands and parents were not available.

Methods

Blood for PAH genotyping was obtained by venipuncture and airmailed at room temperature to The John F. Kennedy Institute, where the genotyping was performed as described previously.5 The general approach was simultaneous amplification of all PAH-coding genomic DNA regions in 13 individual polymerase chain reactions, followed by 1-step scanning for mutations by broad-range denaturing gradient gel electrophoresis9 and direct sequence analysis of samples showing an altered electrophoretic mobility pattern.

Classification of PAH Mutations

Four arbitrary phenotype categories (classic PKU, moderate PKU, mild PKU, and MHP) have been described previously.2,10,11 Here, mutations associated with classic or moderate PKU were collectively assigned as “severe” PKU mutations, resulting in 3 groups for additional analysis. These include mutations that are known or predicted to abolish PAH activity completely (null mutations), as well as mutations that have been identified previously in patients with severe PKU.2,3,12 Mutations that did not fulfill either of these criteria were assigned to 1 of the 2 other phenotype categories on the basis of the phenotypic characteristics of each mutation observed in “functionally hemizygous” patients.2

RESULTS

Blood for genotyping was obtained on 236 probands and their children. A potential causative mutation was identified on 448 (95%) of the 472 mutant PAH alleles, and the complete genotype was ascertained in 214 (91%) of the probands. A total of 84 different mutations were identified, most of which were described in detail in a previous report.3 Additional mutations identified subsequently have also been reported.12

Classification of Mutations

Among the 84 different PAH mutations identified in the present study, 26 are known or predicted to abolish PAH enzyme activity completely and were assigned accordingly as severe PKU mutations. Twenty-one additional mutations were also assigned as severe PKU mutations, because they have been identified previously in patients with severe PKU.3 Among the remaining mutations, 31 could be assigned as either severe or mild PKU, or MHP mutations based on previously observed phenotype characteristics of functionally hemizygous patients.2,3 Six mutations remained unclassified.

According to PAH genotypes, 56% of the 236 probands exhibited severe PKU, 16% exhibited mild PKU, and 16% exhibited MHP. Twelve percent could not be classified.

Relationship Between Genotype and Assigned Phe Level

Complete data on genotype and assigned blood Phe level (APL) were available in 205 probands. Mean APL decreased with increasing severity of genotype (severe PKU: 1622 μmol/L; mild PKU: 1063 μmol/L; MHP: 457 μmol/L). Analysis of variance showed significant differences in APL among these 3 groups (P = 0.0001). Pairwise comparisons were significantly different between all pairs of groups.

Relationship Between Maternal Genotype and Maternal IQ

Among completely classified PKU women, there were 308 pregnancies and 253 live births. Of these, there were 196 mother–offspring pairs with IQ data on both mother and offspring, who are the subjects of the remaining data reported herein. Table 1 shows mean WAIS-R IQ for mothers with PKU having various combinations of mutation classifications and mean WISC-R for their offspring. Analysis of variance showed significant difference in maternal IQ among the groups (P = 0.0001). Mothers with a severe mutation on both PAH alleles demonstrated a mean IQ of 83 (range: 40–116). By comparison, mothers who carried 1 severe mutation and 1 mild mutation demonstrated a mean IQ of 96 (range: 72–125), and mothers who carried a severe mutation and an MHP mutation also showed a mean IQ of 96 (range: 73–130).

Relationship Among Maternal Genotype, Maternal IQ, and Offspring IQ

Cognitive development (WISC-R at 6–7 years of age or later) of 196 offspring was also related to maternal genotype, and there was a significant difference in IQ among offspring of mothers with severe PKU compared with offspring of mothers with mild PKU or MHP (Table 1). Offspring IQ was on average 6 points above maternal IQ. In a non-PKU control group, the offspring also had average IQ scores (WISC-R) 6 points higher than their mothers (P = 0.014). Although the mean IQ values for the offspring were normal, values down to 35 were observed in offspring of mothers with severe PKU. This group of mothers, which comprises approximately two thirds of the investigated population, also showed IQ values down to 40 (Table 1).

For examining the influence of maternal IQ on offspring cognitive development, each of the 3 genotypes could be classified by maternal IQ and offspring IQ. In this study, Table 1 shows the relation between maternal genotype, maternal IQ, and offspring IQ.

TABLE 1. WISC-R IQ for 196 Offspring and WAIS-R IQ for Their Mothers With PKU, Related to Classification of Mutation of Combination (Genotype)

<table>
<thead>
<tr>
<th>IQ</th>
<th>Severe (n = 124; 62%)</th>
<th>Mild (n = 37; 19%)</th>
<th>MHP (n = 35; 19%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Offspring</td>
<td>35–139</td>
<td>65–129</td>
<td>81–128</td>
</tr>
<tr>
<td>PKU mothers</td>
<td>83</td>
<td>96</td>
<td>96</td>
</tr>
<tr>
<td>MHP mutation</td>
<td>40–116</td>
<td>72–125</td>
<td>72–130</td>
</tr>
</tbody>
</table>

Data are means and ranges. Percentage of mothers in each genotype appears in parentheses.
* At age ≤7 years.
type categories was stratified into 2 groups according to maternal IQ: maternal PKU (MPKU) IQ ≥85 and MPKU IQ <85. Offspring of mothers with an IQ ≥85 had a normal mean IQ irrespective of maternal genotype (Table 2). Offspring of mothers with an IQ <85 and a severe genotype had a mean IQ of 83 (range: 35–139). This group comprises 40% of the sample for this report. The IQ range for offspring of mothers with severe genotype and IQ ≥85 was 41 to 133 and thus similar to the range for offspring of mothers with severe genotype and IQ <85 (Table 2).

Although both groups of offspring of mothers with mild PKU (IQ ≥85 vs <85; Table 2) had mean IQs in the normal range, their means differed by 14 points, which is statistically significant (P = .025). The IQ means for the mothers in these 2 groups differed by 21 points, which is also statistically significant (P = 0.025; Table 2). Looking at the total number of 37 mothers with mild PKU and their offspring IQ separately, multiple regression analysis revealed a significant relationship of offspring IQ with maternal IQ (WISC-R mean IQ: 105 and 106; range: 76–139). This group composes 40% of the sample for this report. The IQ range for offspring of mothers with severe genotype and IQ <85 was 41 to 133 and thus similar to the range for offspring of mothers with severe genotype and IQ <85 (Table 2).

Influence of Maternal IQ and Average Phe Exposure During Pregnancy on Cognitive Development in Offspring of Mothers With Severe PAH Genotype

To examine the influence of average Phe exposure during pregnancy on the cognitive development of offspring of mothers with severe PKU, we grouped the sample of 124 mothers with severe PKU and their offspring by MPKU IQ and average Phe exposure: <360 μmol/L, 360 to 750 μmol/L, and >750 μmol/L (Table 3). Both groups of offspring of mothers with average Phe exposure during pregnancy <360 μmol/L had normal cognitive development (WISC-R mean IQ: 105 and 106; range: 76–139) irrespective of maternal cognitive performance (IQ ≥85 vs <85; Table 3). It is noteworthy that offspring of mothers with severe PKU and IQ <85 had a significantly higher WISC-R IQ (mean: 106) compared with

<table>
<thead>
<tr>
<th>Table 2. WISC-R IQ for 196 Offspring and WAIS-R IQ for Their Mothers With PKU, Grouped by Maternal Genotype and Maternal IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Genotype</td>
</tr>
<tr>
<td>Maternal IQ ≥85 (n = 102)</td>
</tr>
<tr>
<td>Offspring IQ</td>
</tr>
<tr>
<td>Maternal IQ</td>
</tr>
<tr>
<td>Offspring IQ</td>
</tr>
<tr>
<td>Maternal IQ &lt;85 (n = 94)</td>
</tr>
<tr>
<td>Offspring IQ</td>
</tr>
<tr>
<td>Maternal IQ</td>
</tr>
<tr>
<td>Offspring IQ</td>
</tr>
</tbody>
</table>

Data are means and ranges.

* P < .01 when compared with maternal IQ.

<table>
<thead>
<tr>
<th>Table 3. WISC-R IQ for Offspring of Mothers With Severe PKU Genotype, Grouped by Average Phe Exposure During Pregnancy and by Maternal IQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal IQ</td>
</tr>
<tr>
<td>Group</td>
</tr>
<tr>
<td>Maternal IQ ≥85 (n = 46; 31%)</td>
</tr>
<tr>
<td>Offspring IQ</td>
</tr>
<tr>
<td>Maternal IQ</td>
</tr>
<tr>
<td>Maternal IQ</td>
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<tr>
<td>Maternal IQ &lt;85 (n = 26; 19%)</td>
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<tr>
<td>Offspring IQ</td>
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<td>Maternal IQ</td>
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<td>Maternal IQ</td>
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<td>Maternal IQ</td>
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</table>

Data are means and ranges. Percentage of mothers in each IQ group appears in parentheses.

P < .0001 when compared with maternal IQ.

P < .005 when compared with maternal IQ.

their mothers’ IQ (WAIS-R mean: 78; P < 0.0001; Table 3). The mothers with severe PKU and IQ <85 might have had a favorable genetic makeup, which was spoiled by poor treatment of PKU during childhood.

When average Phe exposure during pregnancy was >750 μmol/L, offspring IQ was severely depressed (mean IQ: 59 and 54) regardless of maternal IQ (mean IQ: 96 and 74, respectively; Table 3). This group of offspring composes 19% of the group of offspring of mothers with severe PKU.

In offspring of 64 mothers with severe PKU (52% of the group of mothers with this genotype and one third of the entire study population) and an average Phe exposure during pregnancy of 360 to 750 μmol/L, multiple regression analyses showed both maternal IQ (P = 0.0048) and average Phe exposure (P = .0019) to be significant predictors of offspring IQ. The percentage of offspring of mothers with severe PKU and average Phe exposure during pregnancy <750 μmol/L thus is 81%, most of whom were spared mental retardation (Table 3).

Relationship Between Offspring PAH Genotype and Cognitive Development

To examine the effect of offspring PAH genotype on their cognitive development, we stratified IQ data for 157 offspring according to the inherited mutant PAH allele and grouped them by weeks of gestation when metabolic control was established. In offspring who had inherited an MHP allele or a mild PKU allele, mean IQ was relatively intact irrespective of weeks of gestation before metabolic control was established (Table 4). Their mothers must have had either MHP or mild PKU. Eighty-three percent of the offspring who had inherited a severe PKU mutation had mothers with severe PKU. This percentage increased with the time of metabolic control (before conception controlled: 76%; 0–10 weeks until metabolic control was established: 86%; >10–20 weeks: 93%; >20 weeks: 92%). In this group of offspring, mean IQ decreased with delayed maternal Phe control.
offspring (P not significantly different between the 2 groups of type is not a determining factor for offspring IQ. P and maternal Phe (H11005 mean IQ of 78 (range: 59–132; unpublished data). The observation that mothers with severe PKU and a cognitive performance in individuals with PKU. This netic predisposition for cognitive development, eg, 116 suggests that treatment variables may not be the treatment was stopped (7 years vs never). The present IQ range in women with PKU of 40 to 116 suggests that treatment variables may not be the only predictors of cognitive development. The genic predisposition for cognitive development, eg, parent’s IQ, may be another factor influencing cognitive performance in individuals with PKU. This obvious but often neglected factor is illustrated by the observation that mothers with severe PKU and a mean IQ of 78 (range: 59–85) had children with a mean IQ of 106 (range: 76–139) provided that Phe exposure during pregnancy was <360 μmol/L. Fur thermore, when the Phe exposure in pregnant women with severe PKU was kept between 360 and 750 μmol/L, cognitive development in offspring was dependent both on average Phe exposure and on maternal IQ. IQ scores ranged from 35 to 133 in the offspring, indicating a pronounced effect of Phe exposure within this interval. In offspring of mothers with mild PKU, IQ was significantly related to maternal IQ but not to average Phe exposure, which was <750 μmol/L in all cases of mild MPKU. The present observations also suggest a threshold within the interval of 360 to 750 μmol/L, below which no damage occurs and above which there is a linear decline in offspring IQ with increasing maternal Phe. This threshold might be approximately 400 μmol/L.11,13 If maternal Phe is above 750 μmol/L, then offspring IQ is depressed regardless of maternal IQ.

To determine whether offspring genotype had an independent effect on offspring IQ, analysis of covari ance was performed comparing 2 groups of offspring—those with a mild mutation and those with a severe mutation—with maternal IQ and maternal Phe during pregnancy included as covariates. After significant adjustments for maternal IQ (P = .0008) and maternal Phe (P = .0001), mean offspring IQ was not significantly different between the 2 groups of offspring (P = .34), suggesting that offspring genotype is not a determining factor for offspring IQ.

DISCUSSION

The present study shows a decrease in cognitive performance with increasing severity of PAH genotype in adults with PKU. In subjects with severe PKU, mean IQ was 83 (range: 40–116). In a similar Danish population of 47 subjects with severe PKU, mean IQ was 108 (range: 77–132; unpublished data). Among the treatment variables that may account for this difference are mean age when treatment was started (15 months vs <1 month) and age when treatment was stopped (7 years vs never).

The present IQ range in women with PKU of 40 to 116 suggests that treatment variables may not be the only predictors of cognitive development. The genetic predisposition for cognitive development, eg, parent’s IQ, may be another factor influencing cognitive performance in individuals with PKU. This obvious but often neglected factor is illustrated by the observation that mothers with severe PKU and a mean IQ of 78 (range: 59–85) had children with a mean IQ of 106 (range: 76–139) provided that Phe exposure during pregnancy was <360 μmol/L. Furthermore, when the Phe exposure in pregnant women with severe PKU was kept between 360 and 750 μmol/L, cognitive development in offspring was dependent both on average Phe exposure and on maternal IQ. IQ scores ranged from 35 to 133 in the offspring, indicating a pronounced effect of Phe exposure within this interval. In offspring of mothers with mild PKU, IQ was significantly related to maternal IQ but not to average Phe exposure, which was <750 μmol/L in all cases of mild MPKU. The present observations also suggest a threshold within the interval of 360 to 750 μmol/L, below which no damage occurs and above which there is a linear decline in offspring IQ with increasing maternal Phe. This threshold might be approximately 400 μmol/L.11,13 If maternal Phe is above 750 μmol/L, then offspring IQ is depressed regardless of maternal IQ.

It can be concluded that the potential for normal cognitive development is present and will be displayed if treatment is well controlled and continued in childhood and adulthood in female individuals with PKU and that with adequate treatment during pregnancy, they will have children with IQ scores not influenced by their disorder. In the present study, this statement is particularly illustrated by the mothers with a severe PAH genotype.

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