Problem 4. Pathophysiology of the Phenylketonuria

Readings for this problem are found on pages: 79-82, 84, 85-6, 945-6 and 1019 of your Pathophysiology (5th edition) textbook.

(This problem was based on the data from: Y.Okano et al.: Molecular basis of phenotypic heterogeneity of phenylketonuria. New Eng J Med 1991; 324:1232-8).

A five-year old boy was brought by his parents to see a physician because of irregular pronunciation, unusual and very often aggressive behavior and inability to focus his attention towards the contents of the education program in his kindergarten. According to the history, the child was born on time, outside of a hospital and was of the appropriate weight and length. Following previously performed diagnostic tests he was diagnosed with aphasia, deafness and hyperactive syndrome.

The child finds it difficult to construct even the simplest sentences. For example, although he is able to pronounce words like mother, house or a car, the child cannot construct a rudimentary term like “mother’s car”. Psychological tests suitable for the child’s age, showed that his intellectual level measured by IQ was 50-60 (physiological IQ=100±10). The child is of a satisfactory somatic development, light complexion, blond, with blue eyes. Neurological status indicated that the child has clumsy motoric movements, cannot walk on his toes and cannot stand on one leg. The parents negate any mental or neurological disease in the families on both sides. After testing for potential metabolic disorders, the boy was found to have hyperphenylalaninemia combined with mild hypothyrosinemia (Table 1).

Metabolism of phenylalanine is shown in Figure 1. Since hyperphenylalaninemia can occur as a result of impaired pterin metabolism (Fig.1), relevant indicators were analyzed to exclude this possibility (Table 1).

There are more than 50 mutated alleles of phenylalanine-hydroxylase which expressed in homozygote cause hyperphenylalaninemia. The most frequently mutated alleles are listed in Table 2. The patient was found to have alleles R243X and R408W, his father had R408W while mother had R243X. Patient’s brother and sister as well as parents had euphenylalaninemia.

<table>
<thead>
<tr>
<th>Table 1. Laboratory indicators measured in the patient</th>
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<tr>
<td><strong>Measured values</strong></td>
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<tr>
<td>Phenylalanine in plasma</td>
</tr>
<tr>
<td>Tyrosine in plasma</td>
</tr>
<tr>
<td>DHPR-activitya</td>
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<tr>
<td>Bipterin in urine</td>
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<tr>
<td>Phenylpyruvate in urine</td>
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a DHPR = dihydropteridinediole reductase
b J/molHb = unitimol of hemoglobin
Problem 4. Pathophysiology of phenylketonuria

Figure 1. Metabolism of phenylalanine, tyrosine and tryptophan. Only enzymes and substrates relevant for the exercise are shown. ↓ signifies inhibition; dotted lines signify metabolites of phenylalanine produced during hyperphenylalaninemia and secreted by kidneys. The number in a square is a number of metabolites between enzymatic action and final product.
Exercise A: Repetitions of relevant knowledge

1. All of the following claims, pertaining to the phenylalanine metabolism disorder in the patient are correct except for one:

   a) Phenylalanine is an essential amino acid that is used in the child’s early growth and development, approximately 50% for protein synthesis and 50% for synthesis of tyrosine; however, these processes were arrested in the patient due to enzymatic disorder (compare Fig. 1).

   b) Hyperphenylalaninemia occurs as a result of phenylalanine accumulation, following absorption from the chymus, since there is an enzymatic block at the level of phenylalanine-hydroxylase due to a congenital genetic disorder (compare Fig. 1).

   c) Typical phenylketonuria occurs as a result of a reduced phenylalanine-hydroxylase activity below 1% and can develop as a consequence of several different gene mutations (see table 2).

   d) Urine analysis in hyperphenylalaninemia indicates the presence of phenylpyruvate, phenyllactate, phenylacetate and ortho-hydroxyphenylacetate (compare Fig.1); and urine smells like mould.

   e) Protein catabolism in the body releases an excess phenylalanine and that is why the organism is not able to excrete it.
2. All of the following claims, pertaining to the genetically inherited phenylketonuria, are correct except for one:

   a) Monomolecular performing of the phenylalanine-hydroxylase enzymatic function, determines the normal clinical phenotype in the heterozygote (recessive inheritance) since, as in all other enzymopathies, 50 % of functional molecules is enough for a healthy phenotype.
   
   b) Phenylketonuria is a recessive genetically inherited disease that occurs due to a dysfunction of the liver enzyme phenylalanine hydroxylase (compare Fig.1).
   
   c) Newborns are normal at the time of birth but after several months, they start to show nonspecific symptoms like eczema, feeding difficulties and vomiting.
   
   d) Skin, hair as well as urine in the patient can smell like mould because of the phenylpyruvic acid which is produced in an alternative metabolic pathway during hyperphenylalanemia (compare Fig.1).
   
   e) The hair and the skin are hyperpigmented which occurs because the increased concentration of phenylalanine inhibits tyrosinase and causes excessive melanin synthesis (compare Fig.1).

3. All of the following claims, pertaining to the pathogenetic effects of hyperphenylalanemia, are correct except for one:

   a) If there is hyperphenylalanemia (above 1200 µmol/L), it is possible to irreversibly lose about 50 IQ units of intellectual development throughout the first year of development.
   
   b) In adult untreated patients with phenylketonuria, hyperphenylalanemia causes aggressive behavior, increased muscular activity, dysrhythmic EEG readings, however somatic and intellectual status are basically normal.
   
   c) Adult untreated patients with typical phenylketonuria usually have IQ less than 20 and only about 20% of these patients have the IQ exceeding 60.
   
   d) The incidence of hyperphenylalaninemic syndrome is about 1 : 10 000 in European population from which only 2% of the cases result from disrupted pterin metabolism (compare Fig.2 and Table 1).
   
   e) Due to hepatic enzymatic dysfunction hyperphenylalanemia occurs, which causes accumulation of phenylalanine in the neurons that in turn inhibits the myelin synthesis by inhibiting the protein synthesis, and inhibits the synthesis of serotonin as well by inhibiting tryptophan hydroxylase (compare Fig.1).

4. In terms of inheritance and clinical diagnosis of hyperphenylalanemia, it is necessary to consider all of the following facts, except:

   a) In hyperphenylalanemia, the retardation of intellectual development begins already in the second week of postnatal development; however, it is most prominent from the 8th - 9th month of life when it becomes possible to demonstrate pathoanatomical changes in the brain tissue in the form of reduction of white matter and patch-like demyelization.
b) If a patient with hyperphenylalanemia gets pregnant, diffusion of large amounts of phenylalanine into the child’s blood becomes a teratogenic agent that impairs the intrauterine development.

c) In pre-term (i.e. prematurely born newborns) and babies with decreased birth weight (small for gestational age), it is sometimes possible to observe temporary hyperphenylalanemia caused by delayed liver maturation.

d) A gene for phenylalanine hydroxylase is composed of 90 kb forming 13 exons and 12 introns, producing a protein made of 451 amino acids that is expressed only in hepatocytes; the occurrence of nosogenic mutations of this enzyme in European population is approximately 1/50-1/150.

e) In all recessive inherited diseases (including phenylketonuria), due to catalytic and quasi-catalytic molecular activity of the gene product, the nosogenous protein directly inhibits the function of the protein that originates from the normal allele.

5. According to the information provided in introduction, all of the following claims pertaining to the genetic relations in the mentioned family are correct except for one:

a) Since both mother and father are heterozygous, they did not develop hyperphenylalanemia but their children have a 25 % chance to develop the disease.

b) Heterozygotes, based on the fact that they have one healthy allele, have approximately 50% of the normal enzymatic activity which results from codominant gene expression and this level is sufficient to maintain euphenylalaninemia.

c) Since both brother and sister did not develop hyperphenylalanemia, genetically they have one or both normal phenylalanine hydroxylase alleles and consequently a normal phenylalanine metabolism.

d) Since the clinical manifestation of a genetic disease - phenylketonuria can be prevented with a euphenic diet, the patient was unfortunate to have been born outside of a health institution and so his blood sample was not analyzed for hyperphenylalanemia in time.

e) Given the fact that both father and the son have the same nosogenic mutation for phenylalanine hydroxylase (R408W), it can be concluded that the gene is located on the Y chromosome.

Exercise B: Algorhythmic workout of the pathogenesis

Arrange the following terms in causative order:

1. Appearance of phenylpyruvic acid in urine (phenylketonuria) (see Table 1);
2. Hyperphenylalanemia;
3. Mild hypotyrosinemia (see Table 1);
4. Phenylalanine hydroxylase enzyme activity <1%;
5. Father heterozygote with one physiological allele and one R408W mutated allele;
6. R243X mutation inherited from the mother;
7. Inhibition of tyrosine hydroxylase;
8. Inhibited melanin synthesis pathway;
9. Elevated concentration of phenylalanine in neurons;
10. Reduced synthesis of catecholamines;
11. Reduced oxidative transformation of phenylalanine into tyrosine;
12. Inhibited development of serotonergic and catecholaminergic neural pathways;
13. Euphenic diet;
14. Ameliorated neuromotor symptoms;
15. Arrested intellectual development of the child;
16. Arrested myelination of neurons;
17. About 50% of ova are genetically healthy;
18. Patient’s brother and sister are phenotypic healthy individuals;
19. Sister inherited one mutated allele;
20. Physiological concentration of phenylalanine in father’s blood;
21. Inability to construct and pronounce simple sentences;
22. Urine smells like mould;
23. Light complexion and hair;
24. Decreased hyperphenylalanemia;
25. Arrested protein biosynthesis in the neurons;
26. Complex heterozygote (408W/R243X);

Exercise C: Feedback integration of the problem  TYPE V

1. Timely initiated euphenic diet (hypophenylalanine and hypertyrosine diet) can prevent the neuropathophysiologic and psychopathophysiologic changes to accrue, despite the fact that the affected organism will have the genetic deficiency and enzymatic dysfunction for the rest of the life because euphenic diet prevents the development of hyperphenylalanemia (and maintains the blood levels below 484 μmol/L) and thereby blocks the alternate biochemical pathways and inhibits their toxic developmental and symptomatic effects.
   "a"   "b"   "c"   "d"   "e"

2. Toxic effects of hyperphenylalanemia and side products of phenylalanine metabolism on psychomotor development are irreversible since they inhibit the development of serotonergic and catecholaminergic neural pathways as well as myelination (terms 9, 10, 12 and 16 of the algorithm and claim 3e) because severe clinical features of mental retardation occur in untreated patients with high hyperphenylalanemia (>1200 μmol /L), in addition in these patients tyrosine is an essential amino acid and its concentration in plasma depends directly on a dietary intake.
   "a"   "b"   "c"   "d"   "e"
3. Prenatal DNA diagnostics using amniotic fluid cells (see Table 2), allows a
diagnosis of inherited phenylalanine metabolism defect in an otherwise
phenotypically healthy mothers
because
in heterozygotes with a mutated phenylalanine hydroxylase gene, 50% of children
will inherit the mutated gene that is in genetic terms inherited in a recessive
manner.

4. Enzymatic disorders are inherited recessively because of the function performing
mechanism (see claim 2a). How much does the enzymatic activity has to reduce
for the insufficient phenotype and clinical manifestations to appear?

5. By performing blood tests in newborns using either the phenyl-chloride method or
Guthrie bacterial test it is possible to diagnose hyperphenylalaninemia in time.
How many positive test results do you expect during one year in Croatia if there
are about 50 000 births per year (compare claim 3d).