REVIEW

Hyperphenylalaninemia Syndromes: Current Status of Diagnosis and Management

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(Received for publication on June 22, 1987)

Abstract

The phenylalanine hydroxylating system and its related disorders have been intensely investigated during the past decade. This article discusses on some of the newer developments in the study of the physiology and pathophysiology of human phenylalanine metabolism: 1) the phenylalanine hydroxylating system, 2) the early diagnosis of hyperphenylalaninemia syndromes, 3) the molecular basis for phenotypic heterogeneity of phenylketonuria, 4) the prenatal diagnosis of phenylketonuria, and 5) hyperphenylalaninemia caused by defects in tetrahydrobiopterin metabolism.

Key words: PKU, phenylalanine hydroxylase, tetrahydrobiopterin, newborn screening, atypical PKU

In the 50 years since Følling's first description of classical phenylketonuria (PKU), there have been major advances in the understanding of the physiology and pathophysiology of human phenylalanine metabolism. The newborn screening programs for the detection of phenylketonuria, in particular, have enabled us to recognize the extensive clinical and biochemical heterogeneity of hyperphenylalaninemia syndromes. The hyperphenylalaninemias are now classified in terms of deviant hydroxylation components, and various forms of therapeutic strategy have been successfully implemented.

The purpose of this paper is to review newer aspects of the causes, diagnosis, and management of the hyperphenylalaninemia syndromes.

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The phenylalanine hydroxylating system

Although serum phenylalanine concentration is determined by the interaction among various factors including the dietary load, turnover of protein pools, and phenylalanine degradation, no mechanism other than defective phenylalanine hydroxylation accounts for known hyperphenylalaninemic state. In mammals, phenylalanine hydroxylase activity is expressed in liver, kidney, and pancreas, but not in skin fibroblast or peripheral blood cell.¹ The hepatic phenylalanine hydroxylase system, one most extensively studied in humans, consists of 3 essential components, phenylalanine hydroxylase apoenzyme, the coenzyme-tetrahydrobiopterin (BH_4), and dihydropteridine reductase (DHPR). In addition, phenylalanine hydroxylase stimulator (PHS) and dihydrofolate reductase may play subsidiary roles in phenylalanine hydroxylation.²

The first component, phenylalanine hydroxylase, has been purified from rat and human liver. It has a native molecular weight of 100,000 to 110,000, and the K_m for phenylalanine is 0.04 mM, for biopterin 0.003 mM, and for O_2 0.95 percent.^{1,2} The primary role of liver phenylalanine hydroxylase, by virtue of being an allosteric enzyme, is to maintain phenylalanine and tyrosine in tissue at the optimal levels. (In central nervous system, tyrosine is an essential amino acid and a precursor of such biogenic amines as dopamine and norepinephrine.) For this purpose, the enzyme activity is modulated by dual regulation system: the short term regulation by phenylalanine and biopterin, and the long term regulation by changes in the de novo synthesis or degradation of phenylalanine hydroxylase.¹ In short, phenylalanine activates, rather than inhibits, phenylalanine hydroxylase, though the inhibition has been documented with phenylalanine concentration greater than 0.5 mM.

The second component, tetrahydrobiopterin (BH_4), was isolated from the extracts of rat liver as a cofactor for phenylalanine hydroxylase by Kaufman *et al.* in 1963.³ Though biopterin occurs in tetrahydro, dihydro, and fully oxidized forms, most of the biopterin in tissues is believed to be in tetrahydro form.⁴ The major steps in BH_4 biosynthesis have not yet been fully elucidated, but the proposed pathway from guanosine triphosphate (GTP) to tetrahydrobiopterin is outlined in Fig. 1.⁴⁻⁶ There may be, in addition, a methotrexate-sensitive pathway (so-called the salvage pathway) in which dihydrobiopterin (BH_2) is converted to BH_4 by the catalytic action of dihydrofolate reduction.^{1,4} During the hydroxylation reaction, BH_4 is converted to quinonoid BH_2 which is extremely unstable and lacks cofactor activity.

The third component, dihydropteridine reductase, catalyzes a reaction in which the inactive quinonoid BH_2 is regenerated to the active tetrahydroform. The enzyme requires NADH as a cofactor, but NADPH dependent dihydropteridine reductase may also be involved. The enzyme activity is expressed in skin fibroblast and peripheral blood cell as well as in liver and many other tissues.¹

Fig. 1 Biosynthesis of BH₄

The early diagnosis of hyperphenylalaninemia syndromes

The newborn screening program has made it clear that, even with the use of every available laboratory test, one can not unequivocally differentiate those patients who require a diet therapy (classical phenylketonuria) from those who do not (mild hyperphenylalaninemia) in early infancy.⁷ The recognition of hyperphenylalaninemia caused by defects in BH₄ metabolism has further hampered the issue in the differential diagnosis.^{8,9} At present, none is able to present a simplified scheme of a differential diagnosis of hyperphenylalaninemia detected by the newborn screening. Our current strategy, admittedly premature, consists of the following:

1) Every infant with serum phenylalanine level ≥ 10 –20 mg/dl undergoes BH₄ loading test (2 mg/kg/dose, P.O.). If blood phenylalanine level significantly falls, he is temporarily diagnosed as having either a defect in BH₄ synthesis or dihydropteridine reductase deficiency, and urinary neopterin and biopterin are determined by high pressure liquid chromatography (HPLC). In the former, markedly elevated urinary neopterin/biopterin ratio has been invariably found, and in the latter, lowered urinary neopterin/biopterin ratio is expected to be found.^{8,9} If blood phenylalanine level does not appreciably fall, the alternative possibility is either phenylalanine hydroxylase apoenzyme defect or dihydropteridine reductase deficiency.

2) The differentiation between phenylalanine hydroxylase apoenzyme defect and dihydropteridine reductase deficiency depends on urinary pterin patterns. The normal urinary neopterin/biopterin ratio suggests phenylalanine hydroxylase apoenzyme defect, whereas the lowered ratio suggests dihydropteridine reductase deficiency.⁹ If the urinary pterin assays yield equivocal results, the reductase activity needs to be determined (fibroblasts or blood cells).

3) The transiency of hyperphenylalaninemia is to be considered in every infant diagnosed as such. Each infant needs at least monthly check-up including developmental examination, serum aminoacid determination, urine ferric chloride test, and

urine pterine assay, particularly for the first 6 months of life.

In summary, we emphasize that the above scheme is still preliminary, and caution should be exercised with regard to the interpretation of the results. In particular, the age of the patients bears relevance to the appropriate interpretation, since large developmental changes occur in infancy.

Classical PKU and mild hyperphenylalaninemia

Classical PKU and mild hyperphenylalaninemia represent 2 distinctive phenotypes of phenylalanine hydroxylase apoenzyme deficiency.^{1,2} In the former, blood phenylalanine level is markedly elevated ($>1,200 \mu\text{mol/l}$) and phenylalanine hydroxylase activity in the liver is barely detectable ($<1\%$). Ultimately most of untreated patients will have moderately severe mental retardation. In the latter, blood phenylalanine level is only mildly elevated (150 to $800 \mu\text{mol/l}$) and phenylalanine hydroxylase activity is decreased but detectable (2–30%). Without therapeutic intervention, most of the affected children will have normal IQ. It has also been postulated that both disorders are transmitted in autosomal recessive manner: The former represents the homozygous inheritance of 2 classical PKU genes and the latter represents the homozygous inheritance of 2 mild hyperphenylalaninemia genes.

There are, however, a number of hyperphenylalaninemic patients of intermediate severity who do not fit in the framework of the above classification. Some of these intermediate patients may be mentally retarded and are designated as mild PKU. In order to explain an apparent continuum of the hyperphenylalaninemic syndromes, Bartholome *et al.*¹⁰ proposed the hypothesis that a compound heterozygote having one PKU gene and one mild hyperphenylalaninemic gene may yield hyperphenylalaninemia of the intermediate severity. His hypothesis is based upon the assumptions that the gene frequency is 1 to 100 and 1 to 500 for classical PKU and mild hyperphenylalaninemia, respectively. Hardy-Weinberg formula predicts that at least 90% of patients with moderately elevated serum phenylalanine concentration will have the genotype of classical PKU/mild hyperphenylalaninemia. His reasoning, though in part supported by hepatic enzyme activity of biopsied materials, was more or less conceptual.

In 1986, Ledley *et al.* have reported that restriction fragment length polymorphisms (RFLP_s) are associated with various phenylalanine hydroxylase alleles in 2 families in which one member had phenylketonuria and other members had mild hyperphenylalaninemia.¹¹ They have proposed that various combinations of the mutant alleles will result in different phenotypes of the hyperphenylalaninemia syndromes. The correlation between the RFLP haplotype and a specific mutation within phenylalanine hydroxylase locus is not very strong, however. In the families studied, there were several instances in which different mutations of the phenylalanine hydroxylase gene apparently occurred on alleles with the same RFLP haplotype.

In other words, with use of RFLP haplotype as a genetic marker, it is not feasible to identify alleles carrying the mutations of phenylalanine hydroxylase gene or to make meaningful comparisons among the various families with or without the patients. Despite such shortcoming of the RFLP haplotype analysis, the studies of Ledley *et al.* have unequivocally shown that the categorization of mutant phenylalanine hydroxylase alleles as "phenylketonuria genes" or "mild hyperphenylalaninemia genes" is inadequate, and the various combinations of RFLP haplotype primarily determine the phenotypic heterogeneity of classical PKU and mild hyperphenylalaninemia.

We believe that the basis for diverse clinical presentations of PKU and mild hyperphenylalaninemia are probably more complex and multifactorial. We have experienced a Japanese family in which the healthy parents are the first cousins, and elder brother has mild PKU and younger sister has mild hyperphenylalaninemia. Since both subjects are believed to have the same phenylalanine hydroxylase alleles, the different phenotypes of these siblings can not be explained by the theory proposed by Ledley *et al.* The RFLP haplotype analysis of this family is in progress in our laboratory.

Prenatal diagnosis of classical PKU

Phenylalanine hydroxylase activity is not detectable in amniotic fluid cells or chorionic villi trophoblasts.¹ Therefore, PKU can not be diagnosed in utero by a conventional method of the prenatal diagnosis, a measurement of enzymatic activity in the amniotic fluid cells. During the last few years, however, the recombinant DNA technology has increasingly been applied to the prenatal detection of inborn errors of metabolism with an organ-specific enzyme deficiency or without known enzyme abnormality. The following recent developments are relevant to the prenatal diagnosis of classical PKU and related disorders:

1) The human phenylalanine hydroxylase gene has been mapped on the long arm of chromosome 12.¹² 2) The gene, spanning for 90 kb in length with 13 exons, contains all of the necessary genetic information to code for functional phenylalanine hydroxylase.¹³ 3) A full length human phenylalanine hydroxylase complementary DNA (cDNA) has been cloned.¹⁴ 4) Using the phenylalanine hydroxylase cDNA clone as hybridization probe, 10 polymorphic restriction sites have been identified in the phenylalanine hydroxylase locus.¹⁵ 5) The presence or absence of these polymorphic restriction sites has defined RFLP haplotype of the allele. 6) The observed heterozygosity for the RFLP haplotype is 87% in the Danish population.¹⁶ These RFLP haplotypes segregate in a Mendelian manner and concordantly with the mutant alleles (The RFLP and PKU were tightly linked in families studied).

Along a line of such developments, Lidsky *et al.* in 1985 first reported the 2 successful instances in which PKU was diagnosed prenatally by use of RFLP.¹⁷ The RFLP

analysis is an indirect method, however. It does not directly identify a mutant phenylalanine hydroxylase gene and is only applicable to the prenatal diagnosis of families having an affected child. Theoretically, in approximately 90% of PKU families in the caucasian population, the prenatal diagnosis of PKU is possible by RFLP analysis. Unfortunately, similar studies have not been performed in Japanese. It is well conceivable that RFLP haplotypes in the phenylalanine hydroxylase locus are different in Japanese, and the linkage of specific mutations and RFLP haplotypes may not be as tight as in the Danish population.

Hyperphenylalaninemia caused by defects in tetrahydrobiopterin metabolism

During the past decade, at least 3 variants of hyperphenylalaninemia due to BH₄ deficiency have been discovered; GTP cyclohydrolase deficiency, 6-pyruvoyl tetrahydropterin synthase deficiency, and dihydropteridine reductase deficiency. Each variant is characterized by unresponsiveness to phenylalanine restricted diet and severe psychomotor retardation, though there appears to be a remarkable heterogeneity in its clinical manifestation.

GTP cyclohydrolase catalyzes conversion of GTP to D-erythro-7,8-dihydroneopterin 3'-triphosphate and formic acid in the first step of BH₄ biosynthesis. Since the close correlation exists between GTP cyclohydrolase activity and BH₄ availability in various tissues, the enzyme is believed to be of the rate-limiting one.¹ The deficiency of GTP cyclohydrolase was first described in 1984 by Niederwieser *et al.* in a 4-year-old girl who presented at 3 months of age with infantile myoclonic seizure, progressive psychomotor retardation, and repeated bouts of hyperthermia.¹⁸ These investigators showed that her urinary neopterin, biopterin, and several other pterins were depressed, and the hepatic GTP cyclohydrolase was undetectable (<4% of controls).

The patient offered a unique opportunity for Niederwieser *et al.* to assess the previously controversial issues relevant to the basic physiology of the pterins: 1) Are 2 enzymes involved in the conversion of GTP to dihydroneopterin triphosphate? 2) Do neopterin and related compounds play the particular immune functions? Even with extensive work-up neither of these 2 possibilities were positively supported.^{18,19} They showed that the immunoreactive cells of the patient were unable to produce pterins and that all immunological tests were normal. They have also reported that the assay using mononuclear cells can be used for the detection of patients and heterozygous carriers of GTP cyclohydrolase deficiency.

The most common form of BH₄ deficiency, 6-pyruvoyl tetrahydropterin synthase deficiency, was first described in 1978 by Kaufman *et al.*²⁰ The patient was characterized by a progressive neurological deterioration despite the early dietary intervention, and was found to have decreased hepatic tetrahydrobiopterin content, whereas the phenyl-

alanine hydroxylase, dihydropteridine reductase, and dihydrofolate reductase activities were normal. The enzymatic bases for the disorder have still remained nebulous,^{5,21} but the observation that methotrexate did not inhibit the de novo biosynthesis of BH₄ excluded the major role of dihydrofolate reductase in BH₄ biosynthesis.⁵ Thus, dihydrobiopterin and sepiapterin may not be formed as intermediates. The postulated intermediate, 6-pyruvoyl tetrahydropterin is believed to be decreased in a majority of patients with hyperphenylalaninemia due to defective BH₄ biosynthesis, and the designation of 6-pyruvoyl tetrahydropterin synthase deficiency has been proposed.⁶ Niederwieser *et al.* claim that 6-pyruvoyl tetrahydropterin synthase deficiency can be diagnosed enzymatically using erythrocytes as well as liver biopsy.⁶

The results of treatment of this variant have been diverse.⁹ Some patients may be responsive to as low as 2.5 mg/kg/day dose of BH₄, but other patients may not benefit from the doses >20 mg/kg/day. The so-called "peripheral" tetrahydrobiopterin deficiency as characterized by a tetrahydrobiopterin-responsive hyperphenylalaninemia, a high neopterin/biopterin ratio in urine, and normal or elevated concentrations of biopterin, homovanillic acid and 5-hydroxyindole acetic acid in cerebrospinal fluid.^{9,22} The findings suggest that subjects suffer from partial 6-pyruvoyl tetrahydropterin synthase deficiency with sufficient activity to cover the tetrahydrobiopterin requirement in brain but not in liver. Little is known about the optimal mode of therapy for such patients, though many feel that relatively low doses of BH₄ supplement may be justified. Our knowledge on possible side effects of the long term BH₄ and neurotransmitter therapy is also sparse. We have recently reported a 10-year-old Japanese girl with BH₄ deficiency who developed during the course of BH₄ and neurotransmitter treatment marked fluctuations in motor and cognitive performances.²³ Our study has disclosed that Parkinson's disease and BH₄ deficiency share the common underlying mechanisms for the on-off phenomenon, and it is thus well conceivable that similarly affected patients will increase as the survival of such patients is prolonged with the chronic administration of BH₄ and neurotransmitter therapy.

The third variant dihydropteridine reductase deficiency poses difficult problems in diagnosis and therapy.⁹ The disorder was first described by Kaufman *et al.* in 1975–1976, as an unusual form of PKU.^{24,25} They showed that dihydropteridine reductase activity was undetectable in the liver, brain, or cultured skin fibroblasts of the patient. Subsequently, Arai *et al.* reported that the enzyme activity can also be determined on dried blood spots.²⁶ For the clinical diagnosis of dihydropteridine reductase deficiency, 2 procedures, the BH₄ loading test (2 mg/kg/dose, p.o.) and the HPLC determination of urinary neopterin and biopterin, have been commonly used, but neither of the procedures was invariably successful in detecting the entity. In short, about one third of dihydropteridine reductase deficiency patients are non-responders to BH₄, and two thirds are responders. Cotton *et al.* reported that non-responders had the residual mutant dihy-

dropteridine reductase molecules whereas responders had no detectable mutant molecules.²⁷

The absence of characteristic urinary pterin patterns in early infancy was also documented in several patients. Kaufman suspects that dihydrofolate reductase and/or 5,10-methylenetetrahydrofolate reductase may be more active in early infancy, resulting in the regeneration of more BH₄ via alternate pathways.⁹ If his speculation is valid, supplementation with a source of tetrahydrofolate has a place in the treatment of dihydropteridine reductase deficiency. The evidence for the beneficial effect of folinic acid or 5-methyltetrahydrofolate on CNS function is not very strong and needs to be better studied.

Acknowledgements: The author is grateful to Professor Mitsuru Osano for his helpful criticism of the manuscript. This work was partially supported by a Japanese Ministry of Health and Welfare grant (the year of 1986) to N. Matsuo.

References

1. Tourian, A. and Sidbury, J. B.: Phenylketonuria and hyperphenylalaninemia. In: *The Metabolic Basis of Inherited Disease*, 5th ed., edited by Stanbury, J. B., Wyngaarden, J. B., Fredrickson, D. S., Goldstein, J. L. and Broun, M. S., McGraw-Hill, New York, 1983, 270–286
2. Scriver, C. R. and Clow, C. L.: Phenylketonuria: Epitome of human biochemical genetics. *N. Engl. J. Med.* 303: 1336–1342, 1980
3. Kaufman, S.: The structure of the phenylalanine-hydroxylation cofactor. *Proc. Natl. Acad. Sci. USA* 50: 1085–1093, 1963
4. Katoh, S. and Akino, M.: Biosynthesis of tetrahydrobiopterin in animals. *Zool. Sci.* 3: 745–757, 1986
5. Niederwieser, A., Leimbacher, W., Curtius, H. Ch., Ponzone, A., Rey, F. and Leupold, D.: Atypical phenylketonuria with dihydrobiopterin synthetase deficiency: Absence of phosphate-eliminating enzyme activity demonstrated in liver. *Eur. J. Pediatr.* 144: 13–16, 1985
6. Niederwieser, A.: Tetrahydrobiopterin biosynthetic pathway and its deficiency. *Proceedings for The Fourth International Congress of Inborn Errors of Metabolism*, 1987, 26
7. Güttler, F. and Wamberg, E.: On indications for treatment of the hyperphenylalaninemic neonate. *Acta Paediatr. Scand.* 66: 339–344, 1977
8. Dhondt, J. L.: Tetrahydrobiopterin deficiencies: Preliminary analysis from an international survey. *J. Pediatr.* 104: 501–508, 1984
9. Kaufman, S.: Unsolved problems in diagnosis and therapy of hyperphenylalaninemia caused by defects in tetrahydrobiopterin metabolism. *J. Pediatr.* 109: 572–578, 1986
10. Bartholome, K., Olek, K. and Trefz, F.: Compound heterozygotes in hyperphenylalaninemia. *Hum. Genet.* 65: 405–406, 1984
11. Ledley, F. D., Levy, H. L. and Woo, S. L. C.: Molecular analysis of the inheritance of phenylketonuria and mild hyperphenylalaninemia in families with both disorders. *N. Engl. J. Med.* 314: 1276–1280, 1986
12. Lidsky, A. S., Law, M. L., Morse, H. G., Kao, F. T., Rabin, M., Ruddle, F. H. and Woo, S. L. C.: Regional mapping of the phenylalanine hydroxylase gene and the phenylketonuria locus in the human genome. *Proc. Natl. Acad. Sci. USA* 82: 6221–6225, 1985

13. Ledley, F. D., Grenett, H. E., DiLella, A. G., Kwok, S. C. M. and Woo, S. L. C.: Gene transfer and expression of human phenylalanine hydroxylase. *Science* 228: 77-79, 1985
14. Kwok, S. C. M., Ledley, F. D., DiLella, A. G., Robson, K. J. H. and Woo, S. L. C.: Nucleotide sequence of a full-length complementary DNA clone and amino acid sequence of human phenylalanine hydroxylase. *Biochemistry* 24: 556-561, 1985
15. Lidsky, A. S., Ledley, F. D., DiLella, A. G., Kwok, S. C. M., Daiger, S. P., Robson, K. J. H. and Woo, S. L. C.: Extensive restriction site polymorphism at the human phenylalanine hydroxylase locus and application in prenatal diagnosis of phenylketonuria. *Am. J. Human Genet.* 37: 619-634, 1985
16. Daiger, S. P., Lidsky, A. C., Chakraborty, R., Kock, R., Güttler, F. and Woo, S. L. C.: Polymorphic DNA haplotypes at the phenylalanine hydroxylase locus in prenatal diagnosis of phenylketonuria. *Lancet* 1: 299-232, 1986
17. Lidsky, A. L., Güttler, F. and Woo, S. L. C.: Prenatal diagnosis of classic phenylketonuria by DNA analysis. *Lancet* 1: 549-551, 1985
18. Niederwieser, A., Blau, N., Wang, M., Joller, P., Atarés, M. and Cardesa-Garcia, J.: GTP cyclohydrolase I deficiency, a new enzyme defect causing hyperphenylalaninemia with neopterin, biopterin, dopamine, and serotonin deficiencies and muscular hypotonia. *Eur. J. Pediatr.* 141: 208-214, 1984
19. Blau, N., Joller, P., Atarés, M., Cardesa-Garcia, J. and Niederwieser, A.: Increase of GTP cyclohydrolase I activity in mononuclear blood cells by stimulation: detection of heterozygotes of GTP cyclohydrolase I deficiency. *Clin. Chim. Acta* 148: 47-52, 1985
20. Kaufman, S., Berlow, S., Summer, G. K., Milstien, S., Schulman, J. D., Orloff, S., Spielberg, S. and Pueschel, S.: Hyperphenylalaninemia due to a deficiency of biopterin. A variant form of phenylketonuria. *N. Engl. J. Med.* 299: 673-679, 1978
21. Yoshioka, S., Masada, M., Yoshida, T., Mizokami, T., Akiko, M. and Matsuo, N.: Atypical phenylketonuria due to biopterin deficiency: Diagnosis by assay of an enzyme involved in the synthesis of sepiapterin from dihydroneopterin triphosphate. *Zool. Sci.* 1: 74-81, 1984
22. Hreidarsson, S., Valle, D., Holtzman, N., Coyle, J., Singer, H., Kapatos, G. and Kaufman, S.: A peripheral defect in biopterin synthesis: A new mutant. *Pediatr. Res.* 16: 192A, 1982
23. Tanaka, Y., Tsuchiya, Y., Tsuzaki, S., Araki, K. and Matsuo, N.: On-off phenomenon in a 10-year-old Japanese girl with BH_4 deficiency. *Proceedings for The Fourth International Congress of Inborn Errors of Metabolism*, 1987, 122
24. Kaufman, S., Holtzman, N. A., Milstien, S., Butler, I. J. and Krumholz, A.: Phenylketonuria due to a deficiency of dihydropteridine reductase. *N. Engl. J. Med.* 293: 785-790, 1975
25. Milstien, S., Holtzman, N. A., O'Flynn, M. E., Thomas, G. H., Butler, I. J. and Kaufman, S.: Hyperphenylalaninemia due to dihydropteridine reductase deficiency. Assay of the enzyme in fibroblasts from affected infants, heterozygotes, and in normal amniotic fluid cells. *J. Pediatr.* 87: 763-766, 1976
26. Arai, N., Narisawa, K., Hayakawa, H. and Tada, K.: Hyperphenylalaninemia due to dihydropteridine reductase deficiency: diagnosis by enzyme assays on dried blood spots. *Pediatrics* 70: 426-430, 1982
27. Cotton, R. G. H., Jennings, I., Bracco, G., Ponzzone, A. and Guardamagna, O.: Tetrahydrobiopterin non-responsiveness in dihydropteridine reductase deficiency is associated with the presence of mutant protein. *J. Inherited Metab. Dis.* 9: 239-243, 1986