

## Indication Criteria for Genetic Testing *Evaluation of validity and clinical utility*

### Indication criteria for disease: ***Phenylketonuria (PKU) [PAH]***

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## 2. Disease characteristics

2.1 Name of the Disease (Synonyms): *Phenylketonuria*

2.2 OMIM# of the Disease: *261600*

2.3 Name of the Analysed Genes or DNA/Chromosome Segments: *PAH*

2.4 OMIM# of the Gene(s): *612349*

2.5 Mutational Spectrum:

*More than 500 different mutations are known, mostly point mutations (missense, nonsense, splice) but also smaller deletions and duplications. Large deletions and genomic rearrangements are rare (allele frequency approximately 1%, (ref [1]). A large number of the mutations are recorded in the PAH specific database PAHdb (www.pahdb.mcgill.ca). Beware: Apparent homozygosity may be simulated by hemizyosity.*

2.6 Analytical Methods:

*Two different methods are mainly used*  
*-Direct sequencing of genomic exonic DNA with 20bp flanking intronic sequences*  
*-DGGE (a cost-effective and reliable method) (ref [2]) followed by confirmation by direct sequencing.*  
*When two mutations are not identified, a search for partial gene deletions or duplications could be performed by MLPA*  
*Complete analysis of the coding region of the PAH gene should be standard.*

2.7 Analytical Validation

*Combined mutation scanning and sequencing; international proficiency testing (EMQN); in given cases heterozygosity test in parents.*  
*Sequencing of both strands is to be preferred. When a mutation is identified by sequencing the mutation has to be confirmed by re-sequencing of a new PCR product or alternatively confirmed using a second technique (PCR with restriction enzyme digestion), or even better by carrier test in the parents. If the patient is found to be homozygous, carrier test of the parents, or MLPA test is important to exclude hemizyosity. Carrier test of the parents are also able to verify, that the two mutations are present in trans. Participating in EMQN external Quality Assessment for PKU testing is recommended.*

2.8 Estimated Frequency of the Disease in Germany

(Incidence at birth ("birth prevalence") or population prevalence):

*The average incidence at birth (birth prevalence) among Caucasians: 1:10.000 (ref [3]). The incidence in most European countries vary between 1:5000 and 1:15.000 (ref [4])*

2.9 If applicable, prevalence in the ethnic group of investigated person:

*Ireland: 1:4.500 (ref [5]); Turkey 1:2.600 (ref [6]); Japan 1: 143.000 (ref [7]); Africa 1:100.000 (anecdotal, reviewed online; GeneReviews <http://ncbi.nlm.nih.gov/bookshelf>)*

2.10 Diagnostic Setting:

	Yes.	No.
A. (Differential) diagnostics	<input checked="" type="checkbox"/>	<input type="checkbox"/>
B. Predictive Testing	<input type="checkbox"/>	<input checked="" type="checkbox"/>
C. Risk assessment in Relatives	<input checked="" type="checkbox"/>	<input type="checkbox"/>
D. Prenatal	<input checked="" type="checkbox"/>	<input type="checkbox"/>

Comment: *PKU is primarily a biochemical diagnosis; molecular diagnostics is not absolutely required for a good therapy. In special cases (e.g. problematic diet control or marginally elevated phenylalanine blood levels) knowledge of the mutations may clinically be helpful. There is a good correlation between genotype and phenotype; the severity of the phenylalanine hydroxylase deficiency can be predicted from the knowledge of both mutations (ref [8; 9]). The high sensitivity of molecular diagnostics enables detection of heterozygotes in familial risk constellations. Detection of PAH mutations might be important for differential diagnostics of increased blood levels of phenylalanine; 2% of hyper-phenylalaninemias are caused by different types of BH4 deficiency with different molecular aetiology (OMIM: 261630, 261640, 233910, 264070).*

*Prenatal diagnosis might be desirable, especially in poor countries because the diet is expensive. Prenatal diagnosis is possible when the disease causing mutations have been identified in the family. Rules for induced abortion vary in the different European Countries.*

### 3. Test characteristics

		genotype or disease	
		present	absent
test	pos.	A	B
	neg.	C	D

A: true positives      C: false negatives  
B: false positives    D: true negatives

sensitivity:  $A/(A+C)$

specificity:  $D/(D+B)$

pos. predict. value:  $A/(A+B)$

neg. predict. value:  $D/(C+D)$

#### 3.1 Analytical Sensitivity

(proportion of positive tests if the genotype is present)

*Approximately 90% (77%-100%, 95% confidence interval). In 2 genotype positive samples, analyzed at 15 different laboratories, there were 3 genotyping errors (EMQN, Final report for PKU 2008).*

#### 3.2 Analytical Specificity

(proportion of negative tests if the genotype is not present)

*Approximately 93% (80%-100%, 95% confidence interval). In 1 sample analyzed at 15 different laboratories, there was one false positive (EMQN, Final report for PKU 2008).*

#### 3.3 Clinical Sensitivity

(proportion of positive tests if the disease is present)

The clinical sensitivity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

*Approximately 98 % (97%-99%, 95% confidence interval). Using DGGE as screening test, mutations were identified in 99% of 308 PKU alleles from PKU patients from Denmark (97,6%-100%, 95% confidence interval) ([ref2]) and in 97,8% of 438 alleles from PKU patients from Germany (96%-99%, 95% confidence interval) (ref[10]). Using a combination of sequencing and MLPA, mutations were identified in 95% of 66 PKU alleles from PKU patients from Korea (89%-100%, 95% confidence interval) (ref [11]). Beware: 2% of hyperphenylalaninemias are caused by different types of BH4 deficiency with different molecular aetiology (OMIM: 261630, 261640, 264070, 233910). See also 3.1.*

### 3.4 Clinical Specificity

(proportion of negative tests if the disease is not present)

The clinical specificity can be dependent on variable factors such as age or family history. In such cases a general statement should be given, even if a quantification can only be made case by case.

*Only screening of patients with a biochemical diagnosis of PKU (increased level of phenylalanine in the blood) and Heterozygote diagnostics in certain risk constellations (e.g. partner with PKU) are performed. Carriers do not have the disease. See also 3.2.*

### 3.5 Positive clinical predictive value

(life time risk to develop the disease if the test is positive).

*Unknown. Only patients with a biochemical diagnosis of PKU are analysed*

### 3.6 Negative clinical predictive value

(Probability not to develop the disease if the test is negative).

Assume an increased risk based on family history for a non-affected person. Allelic and locus heterogeneity may need to be considered.

Index case in that family had been tested:

*Close to 100% if two typical mutations have been found in the index patient before.*

Index case in that family had not been tested:

*Unknown. Only patients with a biochemical diagnosis of PKU are analysed*

## 4. Clinical Utility

### 4.1 (Differential) diagnosis: The tested person is clinically affected

(To be answered if in 2.10 "A" was marked)

4.1.1 Can a diagnosis be made other than through a genetic test?

No.  (continue with 4.1.4)

Yes, 

clinically.	<input type="checkbox"/>
imaging.	<input type="checkbox"/>
endoscopy.	<input type="checkbox"/>
biochemistry.	<input checked="" type="checkbox"/>
electrophysiology.	<input type="checkbox"/>
other (please describe)	

4.1.2 Describe the burden of alternative diagnostic methods to the patient

*Very low; newborn screening. The phenylalanine blood-level is measured in all newborn.*

4.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?

*The diagnosis of PKU is made primarily by biochemical means. Sometimes the detection of mutations helps in the differential diagnostics of elevated phenylalanine blood levels.*

4.1.4 Will disease management be influenced by the result of a genetic test?

No.

Yes.



Therapy (please describe)

*There is different types of therapy. Phenylalanine restricted diet (ref [12]), treatment with large neutral amino acids (only adults, ref [13]) and treatment with BH4 (patients with mild PKU, ref [14]). A knowledge of the mutations is not absolutely required for good therapy because this is guided by the biochemical findings. In special cases only (e.g. problematic diet control or borderline elevated phenylalanine blood levels) knowledge of the mutations may clinically be helpful. Fluctuating phenylalanine levels are difficult to avoid in classic (severe) PKU, but as a rule, poor compliance is the cause in mild forms of PKU. Knowing the mutations, the metabolic therapist can specifically intervene in such cases. It appears possible that the therapy of PKU with tetrahydrobiopterin (BH4) may profit from a knowledge of the mutations. An adequate evaluation, however, is still to come. Through detection of causative PAH mutations, the clinically and genetically different BH4 deficiency disorders (OMIM: 261630, 261640, 233910) are excluded. (OMIN: 261630, 261640, 233910, 264070) are excluded.*

*Women with PAH deficiency should start a Phe-restricted diet prior to conception and during pregnancy. High amount of phe (>360µmol/l) might lead to abnormalities in the fetus.*

Prognosis (please describe)

*There is a good correlation between genotype and phenotype, the severity of the phenylalanine hydroxylase deficiency can therefore be predicted from the knowledge of both mutations. In cases with marginally elevated phenylalanine levels, knowledge of the genotype may assist in decisions about a possible future need of therapy.(ref [8,9]).*

#### **4.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history**

(To be answered if in 2.10 "B" was marked)

4.2.1 Will the result of a genetic test influence lifestyle and prevention?

If the test result is positive (please describe)

If the test result is negative (please describe)

4.2.2 Which options in view of lifestyle and prevention does a person at-risk have if no genetic test has been done (please describe)?

### 4.3 Genetic risk assessment in family members of a diseased person

(To be answered if in 2.10 "C" was marked)

4.3.1 Does the result of a genetic test resolve the genetic situation in that family?

*As a rule, an affected child with 2 different mutations will be a compound heterozygote, inherited one mutation from each of the parents. Without analysis of the parents, however a novel mutation, occurred only in the child could remain undetected, with the consequence of a false genetic counselling. It may be required in some cases to biochemically exclude a mild hyperphenylalaninemia in one of the parents in order not to miss a third mutation in the family.*

*Heterozygote diagnostics in certain risk constellations (e.g. partner with PKU) is impossible biochemically. Because of the high sensitivity of the screening it can be performed using molecular methods.*

4.3.2 Can a genetic test in the index patient save genetic or other tests in family members?

*No*

4.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?

*Not applicable*

### 4.4 Prenatal diagnosis

(To be answered if in 2.10 "D" was marked)

4.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnostic?

*Yes, but as a rule it is not indicated.*

### 5. If applicable, further consequences of testing

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for the patient or his/her relatives? (Please describe)

*Knowledge of the causative mutations in their child with PKU makes it easier for many parents to deal with a chronically burdensome disease. Specific causal definition of the disease through the genetic changes with the often possible historical classification of mutations (the regional and temporal origin of many mutations is known) is perceived by many parents as positive and helpful for accepting the disease.*

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