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CLINICAL UTILITY GENE CARD

Clinical utility gene card for: Phenylketonuria

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1. DISEASE CHARACTERISTICS

1.1 Name of the disease (synonyms)

Phenylketonuria.

Phenylalanine hydroxylase deficiency that requires treatment.

1.2 OMIM# of the disease

261600.

1.3 Name of the analysed genes or DNA/chromosome segments

PAH.

1.4 OMIM# of the gene(s)

612349.

1.5 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%).[1] A large number of the mutations are recorded in the *PAH*-specific database PAHdb (www.pahdb.mcgill.ca).

1.6 Analytical methods

Two different methods have traditionally been used:

- Direct sequencing of genomic-exonic DNA with at least 20-bp flanking intronic sequences.
- Denaturing gradient gel electrophoresis (DGGE; a cost-effective and reliable method),[2] followed by confirmation by direct sequencing.

DGGE and other mutation scanning methods have now been abandoned by most laboratories. When the analysis fails to identify both mutant alleles, a search for (partial) gene deletions or duplications by multiplex ligation-dependent probe identification (MLPA) may be considered.

Complete analysis of the coding region of the *PAH* gene should be standard; screening for common mutations may be considered in some countries, but should be followed by complete sequence analysis if the analysis fails to identify two mutant alleles.

1.7 Analytical validation

Independent sequencing of both strands of DNA (forward and reverse); combined mutation scanning and sequencing; international proficiency testing (EMQN); in given cases, heterozygosity test in parents.

If the patient is found to be homozygous, carrier test of the parents, or MLPA test is indicated to exclude hemizygosity. In apparent compound heterozygosity, carrier test of the parents is indicated to confirm that the two mutations are present in trans. Participating in EMQN external quality assessment for PKU testing is recommended.

1.8 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)

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

1.9 If applicable, prevalence in the ethnic group of investigated person

Ireland: 1:4.500 (DiLella *et al*[5]); Turkey 1:2.600 (Ozalp *et al*[6]); Japan 1:143.000 (Aoki and Wada[7]); Africa 1:100.000 (Anecdotal, reviewed online [8]).

1.10 Diagnostic setting

	Yes	No
A. (Differential) diagnostics	☒	☐
B. Predictive Testing	☐	☒
C. Risk assessment in relatives	☒	☐
D. Prenatal	☒	☐

Comment:

PKU is primarily a biochemical diagnosis. There is a good correlation between genotype and phenotype; the severity of the phenylalanine hydroxylase deficiency can, in most of the cases, be predicted from the knowledge of the severity of both mutations.[9–11] The severity of a large number of mutations is known.[9,10] Knowing the genotype may be clinically relevant in various circumstances, for example, in children with problematic diet control, or in children with blood phenylalanine levels at the border between PKU (requires treatment) and mild hyperphenylalaninemia (does not require treatment). There is no consensus, regarding the Phenylalanine (Phe) concentration at which treatment should begin. Recommendations varied between different European countries. The level varies from $>200\,\mu$mol/l to $>600\,\mu$mol/l. A Phe level between 200–400 μmol/l is the most reported (42%).[12] Genotype is also clinically relevant for an assessment of possible tetrahydrobiopterin (BH4) cofactor sensitivity. BH4 responsiveness is associated with the genotype. However, patients with

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mutations in the regulatory domain show inconsistent results.[13] Genotyping, by itself, can not be used as a definitive diagnostic test for cofactor sensibility, and it should be combined with a BH4-loading test.[14] 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 hyperphenylalaninemias are caused by different types of BH4 deficiency with different molecular aetiology (OMIM: 261630, 261640, 233910, 264070).

Prenatal diagnosis might be acceptable in countries where treatment is not available (eg, if the cost of treatment is prohibitive). Prenatal diagnosis is possible when the disease-causing mutations have been identified in the family. Rules for induced termination of pregnancy vary in the different European countries.

2. TEST CHARACTERISTICS

	Genotype or disease		A: True positives B: False positives	C: False negative D: True negative
	Present	Absent		
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

2.1 Analytical sensitivity
(proportion of positive tests if the genotype is present)
Approximately 98% in leading laboratories (EMQN scheme: 77–100%, 95% confidence interval; in two genotype positive samples, analysed at 15 different laboratories, there were three genotyping errors; EMQN, final report for PKU 2008; http://www.emqn.org/emqn/Home).

2.2 Analytical specificity
(proportion of negative tests if the genotype is not present)
Approximately 98% in leading laboratories (EMQN scheme: 80–100%, 95% confidence interval; in one sample analysed at 15 different laboratories, there was one false positive; EMQN, final report for PKU 2008; http://www.emqn.org/emqn/Home).

2.3 Clinical sensitivity
(proportion of positive tests if the disease is present)
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),[2] and in 97.8% of 438 alleles from PKU patients from Germany (96–99%, 95% confidence interval).[15] 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).[16] 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.

2.4 Clinical specificity
(proportion of negative tests if the disease is not present)
Only screening of patients with hyperphenylalaninemia (increased level of phenylalanine in the blood) and heterozygote diagnostics in certain risk constellations (eg, partner with PKU) are performed. Carriers do not have the disease. See also 3.2.

2.5 Positive clinical predictive value
(life-time risk of developing the disease if the test is positive)
Individuals with a genotype suggestive of PKU will always have hyperphenylalaninemia on a normal diet. The impact on the clinical phenotype, that is, cognitive development is variable.

2.6 Negative clinical predictive value
(probability of not developing 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.

3. CLINICAL UTILITY

3.1 (Differential) diagnosis: the tested person is clinically affected
(To be answered if in 1.10 'A' was marked)

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

No	☐ (continue with 3.1.4)	
Yes	☒	
	Clinically	☐
	Imaging	☐
	Endoscopy	☐
	Biochemistry	☒
	Electrophysiology	☐
	Other (please describe)	

3.1.2 Describe the burden of alternative diagnostic methods to the patient?
Very low; newborn screening. The phenylalanine blood level is measured in all newborns.

3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?
A genetic test is the ultimate diagnosis; it should always be carried out when possible.

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

No	☐	
Yes	☒	
	Therapy (please describe)	*There are different types of therapy. Phenylalanine restricted diet,[17] treatment with large neutral amino acids (only adults),[18] and treatment with BH4 (patients with mild phenylketonuria).[12] Knowledge of the mutations is not absolutely required for good dietary therapy, because this is guided by the biochemical findings. However, knowing the genotype may be clinically relevant in various circumstances, for example, in children with problematic diet control, or in children with blood phenylalanine levels at the border between phenylketonuria (requires treatment) and mild hyperphenylalaninemia (does not require treatment). Fluctuating phenylalanine levels are difficult to avoid in classic (severe) phenylketonuria, but as a rule, poor compliance*

(Continued)

	is the cause of fluctuating phenylalanine levels in mild forms of phenylketonuria. Knowing the mutations, the metabolic therapist can specifically intervene in such cases. Genotype is also clinically relevant for an assessment of possible tetrahydrobiopterin (BH_4) cofactor sensitivity. Through detection of causative PAH mutations, the clinically and genetically different BH4 deficiency disorders (OMIM: 261630, 261640, 233910, 264070) are excluded. *Women with PAH deficiency should start a Phe-restricted diet before conception and during pregnancy. High amount of Phe (>360 μmol/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, in most of the cases, be predicted from the knowledge of the severity of both mutations.[9,10] In cases with marginally elevated phenylalanine levels, knowledge of the genotype may assist in decisions about a possible future need of therapy.[9,10] Genotype information also provides clue to whether or not tetrahydrobiopterin (BH_4) cofactor sensitivity may be expected, unless the mutation is located in the regulatory domain.[13]*

3.2 Predictive Setting: The tested person is clinically unaffected but carries an increased risk based on family history
(To be answered if in 1.10 'B' was marked)

3.2.1 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):

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

3.3 Genetic risk assessment in family members of a diseased person
(To be answered if in 1.10 'C' was marked)

3.3.1 Does the result of a genetic test resolve the genetic situation in that family?
As a rule, an affected child with two different mutations will be a compound heterozygote, inherited one mutation from each of the parents. Without analysis of the parents, however, a novel mutation that has occurred only in the child could remain undetected, with the consequence of incorrect genetic counselling. It may be required in some cases to biochemically exclude mild hyperphenylalaninemia in one of the parents not to miss a third mutation in the family.

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

3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?
No.

3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?
Not applicable.

3.4 Prenatal diagnosis
(To be answered if in 1.10 'D' was marked)

3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?
Yes, but as a rule it is not indicated.

4. 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.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Birk Møller L, Nygren AO, Scott P *et al*: Low proportion of whole exon deletions causing phenylketonuria in Denmark and Germany. *Hum Mutat* 2007; **28**: 207–211.
2 Guldberg P, Henriksen KF, Güttler F: Molecular analysis of phenylketonuria in Denmark: 99% of the mutations detected by denaturing gradient gel electrophoresis. *Genomics* 1993; **17**: 141–146.
3 Scriver CR, Kaufman S: The hyperphenylalaninemias; in Scriver CR, Beadet AL, Sly SW, Valle D (eds): *The Metabolic and Molecular Bases of Inherited Disease*, 8 edn. McGraw-Hill: New York, 2001, Ch.77.
4 Mathias D, Bickel H: Follow-up study of 16 years neonatal screening for inborn errors of metabolism in West Germany. *Eur J Pediatr* 1986; **45**: 310–312.
5 DiLella AG, Kwok SC, Ledley FD, Marvit J, Woo SL: Molecular structure and polymorphic map of the human phenylalanine hydroxylase gene. *Biochemistry* 1986; **25**: 743–749.
6 Ozalp I, Coskun T, Ceyhan M *et al*: Incidence of phenylketonuria and hyperphenylalaninemia in a sample of the newborn population. *J Inher Metab Dis* 1986; **9**: 237.
7 Aoki K, Wada Y: Outcome of the patients detected by newborn screening in Japan. *Acta Paediatr Jpn* 1988; **30**: 429–434.
8 Anecdotal, reviewed online: GeneReviews http://ncbi.nlm.nih.gov/bookshelf.
9 Guldberg P, Rey F, Zschocke J *et al*: A European multicenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype. *Am J Hum Genet* 1998; **63**: 71–79.
10 Güttler F, Azen C, Guldberg P *et al*: Relationship among genotype, biochemical phenotype, and cognitive performance in females with phenylalanine hydroxylase deficiency: report from the Maternal Phenylketonuria Collaborative Study. *Pediatrics* 1999; **104**: 258–262.
11 Scriver CR, Waters PJ: Monogenic traits are not simple: lessons from phenylketonuria. *Trends Genet* 1999; **15**: 267–272.
12 Blau N, Bélanger-Quintana A, Demirkol M *et al*: Contributing European PKU Centers. Management of phenylketonuria in Europe: survey results from 19 countries. *Mol Genet Metab* 2010; **99**: 109–115.
13 Trefz FK, Scheibe D, Götz H, Frauendienst-Egger G: Significance of genotype in tetrahydrobiopterin-responsive phenylketonuria. *J Inherit Metab Dis* 2009; **32**: 22–26.
14 Feillet F, van Spronsen FJ, MacDonald A *et al*: Challenges and pitfalls in the management of phenylketonuria. *Pediatrics* 2010; **126**: 333–341.
15 Aulehla-Scholz C, Heilbronner H: Mutation spectrum in German patients with phenylalanine hydroxylase deficiency. *Hum Mut* 2003; **21**: 399–400.
16 Lee YW, Lee DH, Kim ND *et al*: Mutation analysis of PAH gene and characterization of a recurrent deletion mutation in Korean patients with phenylketonuria. *Exp Mol Med* 2008; **40**: 533–540.
17 Ahring K, Bélanger-Quintana A, Dokoupil K *et al*: Dietary management practices in phenylketonuria across European centres. *Clin Nutr* 2009; **28**: 231–236.
18 Matalon R, Michals-Matalon K, Bhatia G *et al*: Double blind placebo control trial of large neutral amino acids in treatment of PKU: effect on blood phenylalanine. *J Inherit Metab Dis* 2007; **30**: 153–158.