

Genotype-phenotype correlation in phenylketonuria

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ABSTRACT

Introduction. Phenylketonuria is an inborn metabolism error with a high phenotypical variability, due in part to the large number of implicated genetical variants (over 1200 reported) but also due to other factors. Establishing a genotype=phenotype correlation, accessible today through molecular testing, is an important instrument for diagnostic accuracy, personalized therapy, better evaluation of the prognostic and an optimal genetical advice.

Objective. The article aims to make an analyze of the most recent progress made in the effort of increasing the predictive value of genotyping in establishing the evolution and the severity of the disease.

Material and method. For this review there were analyzed article from specialty journals indexed to Pubmed database, published mainly in the last 10 years.

Results. Genotype-phenotype correlations can be established in most patients, but in approximative 10% of cases there are discordances between previously reported data and the result found in some studies. This mismatch results from the allelic interaction in compound heterozygous, not yet fully understood, from the existence of variants with unpredictable evolution and from other, non-genetical factors.

Conclusions. The genotype-phenotype relationship is increasingly better understood. Molecular testing in phenylketonuria and phenotypical predictions based on the genotype, obtained by comparison with international databases, have clinical importance for the genetic advice given to the family and for the therapeutical decision, among other reasons.

Keywords: phenylketonuria, genotype-phenotype correlation, prediction

INTRODUCTION

Phenylketonuria (PKU) is a genetic disease (MIM #261600) most frequently caused by a mutation in the gene that codes phenylalanine hydroxylase enzyme (PAH; 612349). It is the most frequent inborn error of metabolism [1], with a global incidence of 1:24.000 new-born and an European incidence of 1:10,000 births [2]. The disease is affecting around 450,000 persons [2]. Romania's incidence is situated on the European mean, at 1:10,000 births [3,4].

The hepatic enzyme PAH catalyzes the transformation of the aminoacid phenylalanine (Phe) in tyrosine, in the presence of tetrahydrobiopterin (BH4), oxygen and iron [5]. The variations in the

gene that codes PAH are transmitted autosomal-recessive and lead to lowered PAH enzyme activity and a consecutive accumulation of Phe up to neurotoxic levels [6], with severe consequence for untreated patients, like psychical retardation, behavioral disorders and other psychiatric disorders [1]. Seldom hyperphenylalaninemia is determined by a BH4 deficiency (with normal PAH levels), produced by a biosynthesis or regeneration defect of BH4 [7].

Phenylketonuria is characterized by a broad spectrum of metabolically phenotypes [8]. This is due largely on the high number of genetic variants. There are 1285 known PAH mutations [9], which affect the three PAH domains (regulatory, catalytic and oligomerizator) and include deletions, insertions,

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splicing site variants, missense or nonsense mutations, all with different consequence over the PAH activity [10]. The determinant factor of phenotypic expression in PKU is the residual PAH activity. According to this, PKU is classified in: (i) classic PKU where the enzymatic activity is completely or almost completely abolished, with a plasmatic level of Phe over 1200 $\mu\text{mol/L}$ (20 mg/dl); (ii) mild PKU with a residual PAH activity and plasmatic levels of Phe between 600 and 1200 $\mu\text{mol/L}$ (10-20 mg/dl) [2] and (iii) mild hyperphenylalaninemia with plasmatic PH levels of 120-360 $\mu\text{mol/l}$ (2-6 mg/dl) [2]. Some authors recognize an intermediary form of PKU – moderate PKU – with plasmatic Phe levels of 900-1200 $\mu\text{mol/L}$ (15-20 mg/dl) [11]. The plasmatic levels of Phe are measured pre-treatment. Globally, about one third of the patients have the severe phenotype, classic PKU [2].

The treatment in PKU is based in a large part on the alimentary restriction of Phe, combined with aminoacid supplementation. Some centers use gliomacropetide (GMP) or long chain neutral aminoacids (LNAA) to replace the synthetic aminoacids [7]. Even thaw the dietetical treatment will prevent the installation of mental retardation [12], the existing data suggests that PKU patients have a lower IQ than the control group, even when early diagnosed and treated [13]. In some cases, the treatment with Sapropterin (a synthetic form of the natural cofactor BH4) may lead to an increased Phe tolerance [14]. Genic therapy or enzymatic substitution represent future possible treatments [15,16].

Molecular testing in PKU and phenotype predictions based on the genotype may have clinical importance, especially when the therapeutical recommendations are not clear (ex. Borderline levels of Phe) and is useful when offering the family generic advice [2].

AIM

The aim of this review is to analyze the most recent data from specialized literature which regards the predictive value of the genotype in the metabolic phenotype appreciation of PKU patients. For this there were reviewed 22 articles from Pubmed database, published mainly in the last 10 years. The „pheylketonuria genotype phenotype” keywords were used in the search, and 108 results were obtained. There were excluded from the analyze the irrelevant result (articles that didn't focus on the genotype-phenotype relationship) and also the results that feature only the abstract of the articles.

GENOTYPE-PHENOTYPE RELATION IN PHENYLKETONURIA

The PAH gene, responsible for the synthesis of the PAH enzyme was identify on the human chro-

mosome 12q22–24.1 [1]. It has a length of 90 kb (171 kb if the flanking regions are included), with 13 exons. Although PAH variants may be found practically at any level, the most common places are exon 3, 6, 7 and 11 [10]. Over 60% of mutation are found on the catalytic domain, where the most frequent site is exon 7 [8]. Majority of the variants are missense mutations, followed by deletions, splice site mutations, nonsense mutations and insertions (Figure 1) [8].

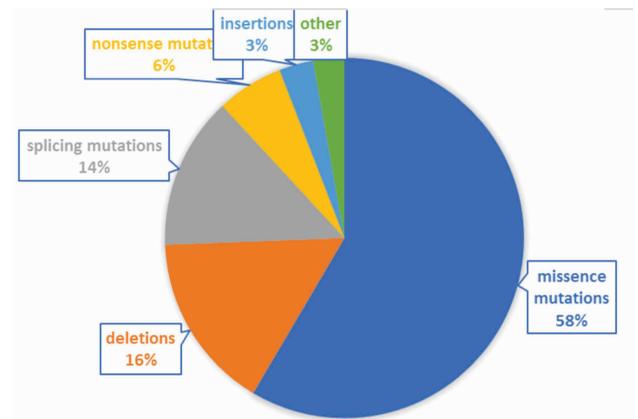


FIGURE 1. PAH variants distribution

The large phenotypical variability in PKU is partially explained by the high number of PAH variants, but not completely. If the enzymatic residual activity is the most important determinant factor in the clinic expression of the disease, and if the residual enzymatic activity is genetically conditioned then the same genetic variant should produce the same effect on the PAH enzyme, and thus the same phenotype [17]. This can be observed in approximate 90% of situations [8]. Many associations between genotype and phenotype were observed (Table 1.) and multiple studies are trying to further add to the research on genotype-phenotype correlations, by using multiple algorithms (ex: FoldX [18], PolyPhen-2 [19], SIFT Blink [20], SNPs3D [21]), with different levels of success [8,22]. There are reported cases where the phenotype expression is discordant, relative to the genotype [23].

TABLE 1. The ten most frequent variants from each PKU phenotypic category [2]

Classic PKU (cPKU)	Milt PKU (mPKU)	HiperPhe non-PKU (MHP)
p.Arg408Trp	p.Tyr414Cys	p.Ala403Val
c.1066-11G>A	p.Arg241Cys	p.Ala300Ser
p.Arg243Gln	p.Arg408Gln	p.Glu390Gly
c.1315+1G>A	p.Arg241His	p.Ile306Val
p.Pro281Leu	p.Arg68Ser	p.Val245Ala
p.Arg158Gln	p.Ala104Asp	p.Asp415Asn
p.Arg252Trp	p.Ala434Asp	p.Val230Ile
c.611A>Ga	p.Ala309Val	p.Thr380Met
p.Glu280Lys	p.Arg261Pro	p.Arg53His
p.Arg261*	c.442-5C>G	p.Glu178Gly

In these circumstances the key in establishing a genotype-phenotype relation is the understanding of some pathological mechanism. Whatever the genetic variant, the reduced protein stability is the principal determinant mechanism of the phenotype, as evidenced by Pey et al. [24] in 2007, and confirmed by later studies [8]. The genetic mutations that occur in the catalytic domain may alter completely the PAH enzyme function [25]. Splice site variants lead to the formation of an truncated, nonfunctional protein [26]. Missence mutations lead to misfolding error of the protein, which will destabilise the protein and oligomer assembly, leading, in the end, to a reduced enzymatic activity [27].

The responsible mechanism for splicing or the mechanism for protein degradation and synthesis are independently transmitted; this is the explanation why people with the same PAH variant may have different phenotypes and why the discordant phenotypes are usually associated with splicing variants [17]. The high discordance rate seen in some cases between the results in some studies and the previously reported data may be due to some unpredictable variants (Ex.: “moderate” variants like p.R261Q, p.V388M or p.I65T and “mild” variants like p.L48S, p.R68S or p.L249F) [22], or due to a classification error resulted from an early analysis (ex.: in the USA the blood spots are taken usually in the first 24-48 hours after birth and the Phe serum levels may not be at maximum value), or due to an individual variation in Phe metabolism [8].

The majority of patients (73% [2]) are compound heterozygous and the allelic interaction can complicate phenotype prediction [8]. With the exception of the situation when both of the allele are null – inactive (with a severe phenotype), in a compound heterozygous situation the milder mutation is always dominant over the severe mutation. [2].

Another often debated subject in the literature is the level of response to Sapropterin treatment. BH4 is involved in the process of protein folding, contributing at the enzyme stabilization and preventing its degradation [5]. In the situation of a residual enzymatic activity, BH4 enhances PAH activity, resulting a greater tolerance for Phe and implicit a greater quality of life [7,28]. The response at BH4 therapy seems to be dependent of the pretreatment plasmaic Phe levels; a minimum residual activity is required [29]. In 79-83% of cases of HPA, 49-60% of mild PKU and 7-19 % of classic PKU [29], patients may benefit from BH4 treatment [17,30], while the

association of two null variants is correlated with lack of response to BH4.

Phenotype prediction by genotype determination concerning the response to BH4 had been obtained in three quarters of subjects in an 2015 study [8]. Many of the missence PAH variants in the oligomerizing domain which maintain a residual enzymatic activity are correlated with a good response at BH4 therapy [30].

The understanding of genetic fundamentals which stand at the basis of the inborn metabolism errors is an ongoing endeavor. Despite of the high volume of data obtain by the continuous research process, associations between genotype and phenotype are not always possible. In a lot of genetic maladies, including phenylketonuria, the low number of reported cases limits the capacity of genotype-phenotype correlations. The interpretation of data becomes even more complicated when taken in consideration the proteomics and epigenomics factors.

In phenylketonuria, genotyping represents an efficient method of phenotype prediction, with a 89% accuracy [8]. The prediction is much simpler when the genotype is represented by the association of two null variants, in this situation the phenotype is a severe one – classic PKU, but the prediction can become complicated in some situations. The complexity in PKU is due to the large number of genetic variants, allelic interactions in compound heterozygous and due to non-genetic factors, that can influence the diagnosis and the disease evolution.

Genotype based prediction of metabolic phenotype is useful in the management of hyperphenylalaninemia. Also, in a good degree (approx. 75% of cases [8]) the response to BH4 treatment may be anticipated by genotype determination.

CONCLUSIONS

Once genomic sequencing became accessible, the genotype-phenotype relationship is better understood in more and more genetic disorders (including PKU).

In practice, PKU genotyping has an important role as a predictive factor, helping both in therapeutic decision and in genetic advice.

The metabolic phenotype and phenylalanine tolerance anticipation leads to a better, personalized therapeutic approach and a superior quality of life.

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