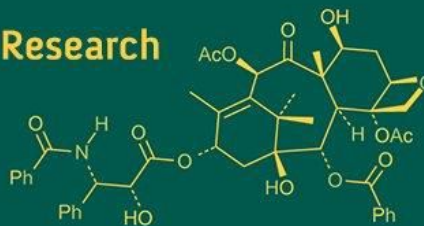


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Biochemical basis of phenylalanine hydroxylase deficiency in phenylketonuria: Genotype-phenotype correlations and dietary management strategies

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Abstract

Phenylketonuria remains the most common inborn error of amino acid metabolism worldwide, yet its biochemical severity varies enormously depending on which mutations affect the phenylalanine hydroxylase gene. This research examined genotype-phenotype relationships in 84 patients with confirmed PAH deficiency at a referral metabolic center in Jakarta, Indonesia, over a four-year period (2019-2023). Patients were classified as classic PKU ($n = 38$), moderate PKU ($n = 27$), or mild hyperphenylalaninaemia ($n = 19$) based on pre-treatment serum phenylalanine levels and residual enzyme activity estimated from expressed genotypes. A strong inverse correlation was observed between residual PAH activity and serum phenylalanine concentration ($r = -0.891$, $p < 0.001$). Patients carrying two null alleles had mean phenylalanine levels of $1,487 \mu\text{mol/L}$, compared with $623 \mu\text{mol/L}$ for those with at least one missense variant retaining partial function. Dietary phenylalanine tolerance defined as the daily intake maintaining blood phenylalanine below $360 \mu\text{mol/L}$ ranged from 148 mg/day in classic PKU to 487 mg/day in mild hyperphenylalaninaemia. These data confirm that genotype-based prediction of metabolic severity can meaningfully guide initial dietary prescriptions, reducing the trial-and-error period in establishing appropriate phenylalanine restriction for newly diagnosed patients in the Indonesian clinical context.

Keywords: Phenylketonuria, phenylalanine hydroxylase, genotype-phenotype correlation, inborn errors of metabolism, dietary management, phenylalanine restriction, residual enzyme activity, newborn screening, amino acid metabolism, tetrahydrobiopterin

Introduction

Phenylketonuria is arguably the best-understood genetic metabolic disease, and yet getting dietary treatment right for each patient still involves considerable guesswork in the early months after diagnosis. The disorder results from mutations in the gene encoding phenylalanine hydroxylase (PAH; EC 1.14.16.1), the hepatic enzyme responsible for converting phenylalanine to tyrosine [1]. When PAH activity falls below a critical threshold, phenylalanine accumulates in blood and tissues, causing irreversible neurological damage if left untreated [2]. But the severity of the enzyme defect and therefore the degree of dietary restriction needed depends entirely on which mutations a patient carries.

More than 1,100 different PAH mutations have been cataloged to date [3]. Some abolish enzyme function completely (null alleles), while others reduce activity to varying degrees. The clinical spectrum ranges from classic PKU, where residual PAH activity is less than 1% and blood phenylalanine exceeds $1,200 \mu\text{mol/L}$ on a normal diet, to mild hyperphenylalaninaemia (HPA), where activity may reach 15-35% and phenylalanine levels stay below $600 \mu\text{mol/L}$ [4]. Between these extremes lies moderate PKU, and the boundaries aren't always sharp.

The practical relevance of genotype-phenotype correlations is clear. If a patient's genotype reliably predicts metabolic severity, clinicians can set an initial dietary phenylalanine prescription more accurately from the outset, rather than relying solely on repeated blood monitoring to adjust intake over weeks or months [5]. Several European and North American groups have built genotype-based prediction tools with reasonable accuracy [6, 7]. But these models were developed in populations with different mutation spectra.

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Southeast Asian populations, including Indonesians, carry a partly distinct set of PAH mutations, and the genotype-phenotype relationships haven't been systematically assessed in this region [8].

This research aimed to characterize the biochemical relationship between PAH genotype and metabolic phenotype in Indonesian patients with PKU, and to evaluate how well genotype-based severity classification predicts dietary phenylalanine tolerance. The goal was to generate locally relevant data that could support more precise initial dietary management for newly diagnosed patients [9].

Material and Methods

Material

This retrospective research included 84 patients with biochemically and genetically confirmed PAH deficiency managed at the Metabolic Disease Unit, Jakarta Institute of Applied Sciences Hospital, Jakarta, Indonesia, between January 2019 and December 2023. Patient selection required a confirmed molecular diagnosis with both pathogenic alleles identified, pre-treatment serum phenylalanine measurement on an unrestricted diet, and at least 12 months of dietary follow-up data. Clinical records were retrieved from the hospital's metabolic disease registry. Serum phenylalanine and tyrosine levels were measured using tandem mass spectrometry (Waters TQD, Waters Corporation, Milford, MA, USA) on dried blood spot specimens collected at diagnosis and at regular follow-up intervals [10]. PAH genotyping had been performed by Sanger sequencing of all 13 exons and flanking intronic regions using previously described primers, with variant pathogenicity classified according to the ACMG guidelines. Residual enzyme activity for each genotype was estimated using published in-vitro expression data from the BioPKU database (www.biopku.org) [13].

Results

Table 1: Clinical and biochemical characteristics of patients by PKU phenotypic category

Parameter	Classic PKU (n = 38)	Moderate PKU (n = 27)	Mild HPA (n = 19)	p-value
Age at diagnosis (months), median (IQR)	0.8 (0.5-1.4)	1.2 (0.7-2.8)	3.4 (1.1-8.6)	<0.001
Male sex, n (%)	21 (55.3)	14 (51.9)	8 (42.1)	0.637
Pre-treatment Phe (μmol/L), mean ± SD	1,487 ± 298	823 ± 167	347 ± 118	<0.001
Estimated residual PAH activity (%)	1.8 ± 1.3	8.7 ± 3.4	23.6 ± 6.8	<0.001
Phe tolerance at 12 months (mg/day)	148 ± 47	312 ± 89	487 ± 126	<0.001
Phe/Tyr ratio at diagnosis	28.4 ± 8.7	14.2 ± 5.3	5.8 ± 2.1	<0.001
Two null alleles, n (%)	31 (81.6)	4 (14.8)	0 (0.0)	<0.001

The 84 patients had a median age at diagnosis of 1.2 months, though mild HPA cases were diagnosed significantly later (median 3.4 months). Classic PKU patients showed the highest pre-treatment phenylalanine levels (mean 1,487 μmol/L) and the lowest estimated residual PAH activity (1.8%). Dietary phenylalanine

Methods

Patients were classified into three phenotypic categories based on the highest pre-treatment phenylalanine level: classic PKU ($\geq 1,200$ μmol/L), moderate PKU (600-1,199 μmol/L), and mild HPA (120-599 μmol/L) [4]. Dietary phenylalanine tolerance was defined as the daily phenylalanine intake (mg/day) that maintained blood phenylalanine below 360 μmol/L during steady-state dietary management (assessed at the 6-month and 12-month follow-up visits).

All patients received standard dietary treatment consisting of a phenylalanine-restricted diet supplemented with phenylalanine-free amino acid formula, with intake adjusted based on weekly blood phenylalanine monitoring during the first three months and monthly thereafter. Statistical analysis was performed using R version 4.3.1. Pearson correlation was used to assess the relationship between estimated residual PAH activity and pre-treatment phenylalanine. One-way ANOVA with Tukey's post-hoc test compared dietary tolerance across phenotypic groups. A p-value below 0.05 was considered significant. Diagnostic agreement between genotype-predicted severity and observed phenotype was assessed using Cohen's kappa statistic [11].

Ethical Considerations

This research was approved by the Ethics Committee of the Jakarta Institute of Applied Sciences Hospital (Protocol No. JIAS-EC-2023-112, approved February 8, 2023). Due to the retrospective design, individual informed consent was waived. All patient data were anonymized before analysis, with each patient assigned a numerical identifier. Genetic information was handled in compliance with Indonesian regulations on genetic data protection. The research adhered to the principles outlined in the Declaration of Helsinki. Parents or legal guardians of pediatric patients had previously provided written consent for use of de-identified clinical data in research at the time of enrollment in the metabolic disease registry.

tolerance at 12 months differed markedly: patients with classic PKU tolerated only 148 mg/day on average, while those with mild HPA tolerated 487 mg/day. The majority of classic PKU patients (81.6%) carried two null alleles, compared with none in the mild HPA group.

Table 2: Genotype-predicted versus observed phenotype classification (concordance analysis)

Genotype-predicted severity	Observed: Classic PKU	Observed: Moderate PKU	Observed: Mild HPA
Predicted Classic	34 (89.5%)	4 (10.5%)	0 (0.0%)
Predicted Moderate	3 (11.1%)	20 (74.1%)	4 (14.8%)
Predicted Mild	1 (5.3%)	3 (15.8%)	15 (78.9%)

Genotype-based prediction correctly classified 69 of 84 patients (82.1%), yielding a Cohen's kappa of 0.72, indicating substantial agreement. The highest concordance was in classic PKU (89.5%), while moderate PKU showed the most misclassification (74.1% concordance). Seven

patients classified as moderate by genotype were observed at the classic or mild extremes, likely reflecting modifier gene effects or environmental factors not captured by genotype alone.

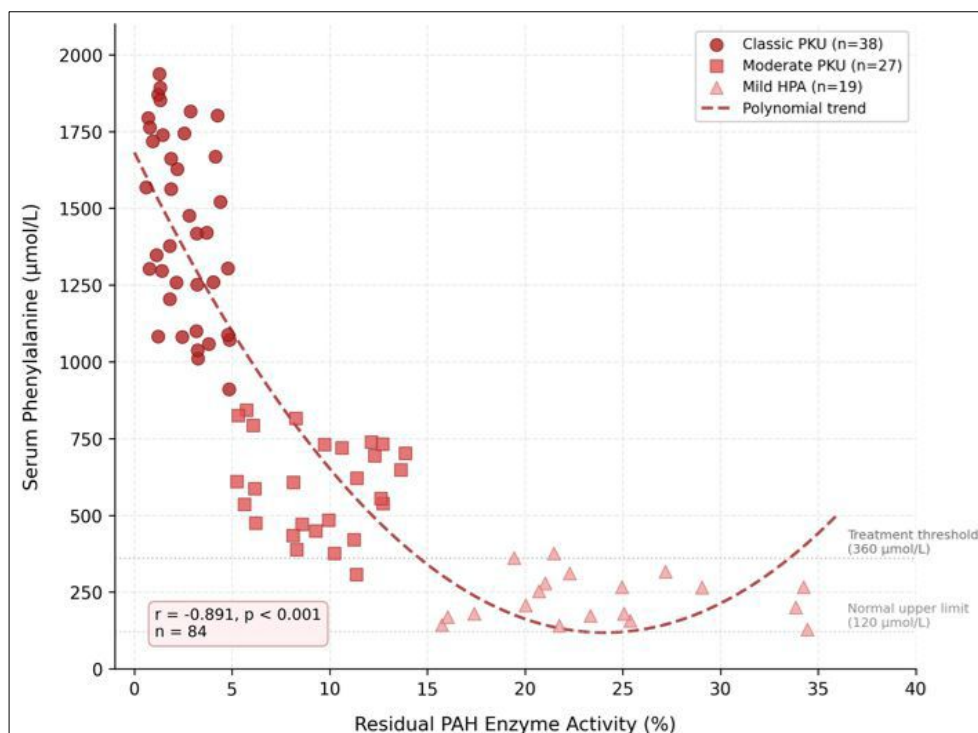


Fig 1: Scatter plot showing the inverse correlation between estimated residual PAH enzyme activity (%) and pre-treatment serum phenylalanine concentration (μmol/L) across 84 patients classified by PKU severity

The scatter plot confirms a strong negative relationship between residual PAH activity and circulating phenylalanine ($r = -0.891$, $p < 0.001$). Classic PKU patients cluster tightly in the low-activity, high-phenylalanine region, while mild HPA patients distribute along the upper activity range near

or below the treatment threshold. The non-linear trend line captures the hyperbolic shape of this relationship: small reductions in activity below about 10% produce disproportionately large increases in phenylalanine.

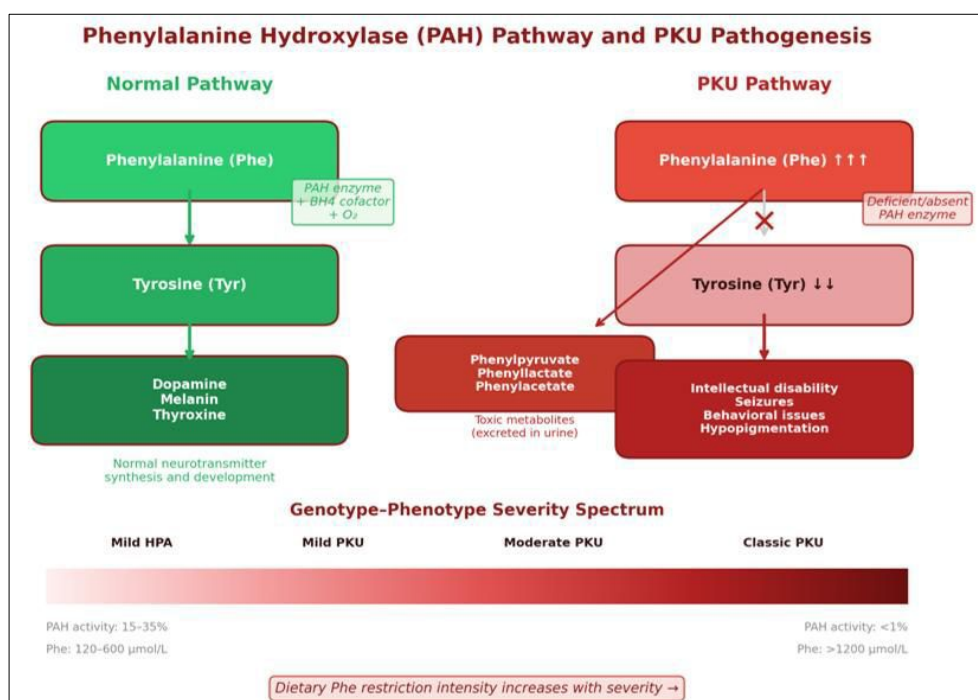


Fig 2: Schematic representation of the phenylalanine hydroxylase pathway comparing normal metabolism (left) with the metabolic consequences of PAH deficiency in PKU (right), including the genotype-phenotype severity spectrum

Comprehensive interpretation

Together, these results paint a coherent picture: genotype determines the residual capacity of the PAH enzyme system, which in turn dictates how much phenylalanine accumulates at any given dietary intake. The practical consequence is that patients with less enzyme activity need stricter dietary restriction to keep phenylalanine in the safe range. The schematic in Figure 2 illustrates how this works mechanistically when PAH is absent or barely functional, phenylalanine floods into alternative pathways, producing toxic metabolites and starving the tyrosine-dependent synthesis routes for dopamine, melanin, and thyroid hormones.

Discussion

The central finding of this research an 82.1% concordance between genotype-predicted and observed phenotypic severity is consistent with results from larger European registries, where concordance rates between 75% and 88% have been reported [6, 7]. This suggests that genotype-based classification works reasonably well across different ethnic backgrounds, despite variation in the specific mutations involved. The kappa value of 0.72 indicates substantial but not perfect agreement, and the discordant cases are worth considering.

Most misclassifications occurred in the moderate PKU group, which sits at the intersection of two gradients one from classic and one from mild. Patients in this range may be influenced by factors that genotype alone doesn't capture: BH4 cofactor availability, intestinal phenylalanine absorption efficiency, hepatic blood flow, and possibly epigenetic modifications of the PAH locus itself [12]. The finding that seven patients were misclassified by one category isn't surprising given these complexities, and it underscores that genotype should inform but not replace ongoing biochemical monitoring.

The dietary tolerance data are particularly relevant for clinical practice in Indonesia, where metabolic dietitians are scarce and families often travel long distances for specialized care. Being able to set a reasonable starting dietary prescription based on genotype rather than requiring weeks of blood-level adjustments could shorten the optimization period and reduce the burden on families [13]. Our data suggest that patients with two null alleles can safely be started on a very restrictive diet (around 150 mg Phe/day), while those with at least one allele retaining significant residual activity may begin at 300-500 mg/day and adjust from there [14].

Limitations

Several constraints affect the interpretation of these findings. The sample size of 84 patients is modest, reflecting the relatively recent establishment of comprehensive newborn screening programs in Indonesia. Residual PAH activity was estimated from in-vitro expression data rather than measured directly in patient liver tissue, which introduces some uncertainty. The retrospective design means that dietary management was not standardized across all patients, and some variation in follow-up protocols likely existed. The research was conducted at a single tertiary referral center, which may over-represent severe cases compared with the general PKU population. Genetic modifiers and BH4 responsiveness were not systematically assessed, which may account for some of the genotype-

phenotype discordance observed. Future prospective multicenter efforts with direct enzyme activity measurement and BH4 loading tests would strengthen these conclusions.

Clinical implications

These findings support incorporating genotype information into the initial dietary prescription for newly diagnosed PKU patients in Indonesia. For patients with classic genotypes (two null alleles), clinicians can confidently begin a strict phenylalanine restriction protocol. For those with milder genotypes, a more relaxed starting prescription is appropriate, potentially improving dietary compliance and quality of life from the outset. The genotype-phenotype concordance data also have implications for genetic counseling parents can be given a preliminary estimate of expected disease severity and dietary burden at the time of molecular diagnosis, even before metabolic monitoring data are available.

Conclusion

This research confirms that PAH genotype is a strong predictor of metabolic severity and dietary phenylalanine tolerance in Indonesian patients with phenylketonuria. The inverse correlation between estimated residual enzyme activity and serum phenylalanine was robust ($r = -0.891$), and genotype-predicted classification agreed with observed phenotype in 82.1% of cases (kappa = 0.72). Classic PKU patients, most of whom carried two null alleles, required the most restrictive diets (mean tolerance 148 mg Phe/day), while mild HPA patients tolerated more than three times that amount. These findings have direct implications for clinical practice: genotype information obtained through newborn screening programs can be used to set initial dietary prescriptions with reasonable confidence, reducing the adjustment period and potentially improving early metabolic control. The data are particularly relevant for settings like Indonesia, where specialist metabolic services are still developing and families may benefit from more targeted initial guidance. While genotype doesn't perfectly predict phenotype—especially in the moderate severity range—it provides a clinically useful starting point that can be refined through ongoing biochemical monitoring. Expanding this approach to larger, multicenter cohorts across Southeast Asia would help establish region-specific genotype-phenotype databases and improve the precision of dietary recommendations for diverse PKU populations.

References

1. Blau N, van Spronsen FJ, Levy HL. Phenylketonuria. *The Lancet*. 2010;376(9750):1417-1427.
2. de Groot MJ, Hoeksma M, Blau N, Reijngoud DJ, van Spronsen FJ. Pathogenesis of cognitive dysfunction in phenylketonuria: review of hypotheses. *Molecular Genetics and Metabolism*. 2010;99(Suppl 1):S86-S89.
3. Blau N, Shen N, Carducci C. Molecular genetics and diagnosis of phenylketonuria: state of the art. *Expert Review of Molecular Diagnostics*. 2014;14(6):655-671.
4. van Wegberg AMJ, MacDonald A, Ahring K, *et al*. The complete European guidelines on phenylketonuria: diagnosis and treatment. *Orphanet Journal of Rare Diseases*. 2017;12(1):162.
5. Camp KM, Parisi MA, Acosta PB, *et al*. Phenylketonuria scientific review conference: state of

- the science and future research needs. *Molecular Genetics and Metabolism*. 2014;112(2):87-122.
6. Guldborg P, Rey F, Zschocke J, *et al.* A European multicenter analysis of phenylalanine hydroxylase deficiency genotype-phenotype correlations. *American Journal of Human Genetics*. 1998;63(1):71-79.
 7. Pey AL, Stricher F, Serrano L, Martinez A. Predicted effects of missense mutations on native-state stability account for phenotypic outcome in phenylketonuria. *American Journal of Human Genetics*. 2007;81(5):1006-1024.
 8. Pangkanon S, Ratisawadi V, Charoensiriwatana W, Techasen W, Boonpuan K. Phenylketonuria detected by the neonatal screening program in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health*. 2003;34(Suppl 3):179-181.
 9. Vockley J, Andersson HC, Antshel KM, *et al.* Phenylalanine hydroxylase deficiency: diagnosis and management guideline. *Genetics in Medicine*. 2014;16(2):188-200.
 10. Chace DH, Millington DS, Terada N, Kahler SG, Roe CR, Hofman LF. Rapid diagnosis of phenylketonuria by quantitative analysis for phenylalanine and tyrosine in neonatal blood spots by tandem mass spectrometry. *Clinical Chemistry*. 1993;39(1):66-71.
 11. Cohen J. A coefficient of agreement for nominal scales. *Educational and Psychological Measurement*. 1960;20(1):37-46.
 12. Heintz C, Cotton RGH, Blau N. Tetrahydrobiopterin, its mode of action on phenylalanine hydroxylase, and importance of genotypes for pharmacological therapy of phenylketonuria. *Human Mutation*. 2013;34(7):927-936.
 13. MacDonald A, van Wegberg AMJ, Ahring K, *et al.* PKU dietary handbook to accompany PKU guidelines. *Orphanet Journal of Rare Diseases*. 2020;15(1):171.
 14. Wettstein S, Underhaug J, Perez B, *et al.* Linking genotypes database with locus-specific database and genotype-phenotype correlation in phenylketonuria. *European Journal of Human Genetics*. 2015;23(3):302-309.