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Beyond the Screening Cut-off: Measurement Drift, Predictors and Consanguinity in Borderline Neonatal Phenylalanine Among More Than 500,000 Newborns

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Research Article

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Abstract

Background

Newborn screening identifies many infants with phenylalanine above the upper limit of normal (2 mg/dL; 120 μmol/L) but below the treatment threshold (6 mg/dL; 360 μmol/L). This borderline group is far larger than the treatment-range group but has rarely been characterized in unselected populations.

Methods

We analyzed a regional registry (Mashhad University of Medical Sciences, northeastern Iran) of 543,952 children with a newborn screening phenylalanine value, classified as normal (< 2 mg/dL), borderline (2 to < 6 mg/dL) or treatment-range (≥ 6 mg/dL). We assessed year-to-year measurement drift, identified predictors of borderline status using logistic regression adjusted for birth year and repeated the analyses after harmonizing values across years, compared associations with parental consanguinity between categories, and examined weight- and length-for-age at birth.

Results

Borderline values were recorded in 10.5% of children, but this proportion ranged from 1.1% to 25.5% across birth years because of a parallel upward shift in the whole phenylalanine distribution that was not explained by child characteristics and was absent for TSH measured in the same blood spot; after harmonization, 1.1% of children had borderline values. Treatment-range values occurred in 63 children (11.6 per 100,000). Preterm birth (OR 1.20, 95% CI 1.15–1.26) predicted borderline status under every definition, and female sex (OR 1.09, 1.07–1.12) under all definitions covering the whole study period. Parental consanguinity was not associated with borderline status (OR 1.00, 0.98–1.03) but was associated with treatment-range values (OR 3.19, 1.92–5.30; difference $p < 0.001$), including after harmonization. Borderline phenylalanine was associated with slightly lower weight-for-age at birth, also in term singletons (− 0.06 SD, 95% CI − 0.07 to − 0.05).

Conclusions

Much of the apparent borderline population in this registry reflected measurement drift rather than differences between children. After accounting for it, borderline elevation had modest perinatal predictors and no association with consanguinity, unlike treatment-range hyperphenylalaninemia, suggesting that the two groups are largely distinct. Screening programs should monitor analytical drift, and registry-based studies should account for it.

Background

Phenylketonuria (PKU) is an autosomal recessive disorder of phenylalanine metabolism, caused principally by deficiency of phenylalanine hydroxylase, that leads to toxic accumulation of phenylalanine unless detected early and managed with a phenylalanine-restricted diet [1, 2]. Newborn screening for PKU, first introduced in the United States in the 1960s and soon followed by national programs elsewhere, including the United Kingdom [3], is now among the most widespread public health screening programs globally, and has transformed classical PKU from a leading cause of preventable intellectual disability into a manageable chronic condition when detected early [4, 5], though affected individuals continue to require lifelong monitoring for metabolic and comorbid health outcomes [6]. Screening identifies a spectrum of phenylalanine elevation, conventionally divided into classical/severe PKU, mild-to-moderate PKU, and mild hyperphenylalaninemia (phenylalanine above the upper limit of normal of about 120 μmol/L [2 mg/dL] but below 360 μmol/L [6 mg/dL], generally not requiring dietary treatment) [7]. A much larger group of infants have borderline values at the initial screen, most of whom have transient elevation or normal values on repeat testing; this group far outnumbers those in the treatment range but has received little attention because it does not trigger treatment.

PKU incidence varies substantially by population, ranging from approximately 1 in 2,600 in Turkey to roughly 1 in 20,000 in some Arab Gulf states, a pattern attributed in large part to differences in consanguineous marriage rates given the condition's autosomal recessive inheritance [8]. Iran-specific estimates likewise vary widely by region: a national systematic review and meta-analysis pooling 18 studies and over 3.3 million Iranian neonates estimated overall PKU prevalence at 16.5 per 100,000 live births (95% CI 12.9–21.2), with suspected hyperphenylalaninemia at 45.6 per 100,000 and classical PKU specifically at 4.4 per 100,000 [9]. Regional Iranian estimates have ranged from as low as 5.7 per 100,000 in northeastern Iran (2012–2013) to substantially higher rates in some southern provinces, where one district-level study in Fars province reported a prevalence as high as 1 in 382 births, accompanied by a parental consanguinity rate of 86.6% among affected families [10]. This heterogeneity underscores the value of population-specific, contemporary prevalence estimates.

Beyond diagnosis and prevalence, the relationship between phenylalanine level and childhood physical growth remains incompletely characterized, particularly outside the setting of formally diagnosed and dietetically managed PKU. Among children with confirmed PKU, growth has historically been reported as reduced from birth onward under early, stringent phenylalanine-restricted diets, though this pattern has attenuated in more recent cohorts with advances in protein-substitute formulations [11]. Within phenylalanine-restricted populations, growth trajectories differ by degree of dietary control: children with well-controlled phenylalanine levels show more favorable height and weight trajectories than those with poorly controlled levels [12], consistent with evidence-based nutrition management guidelines emphasizing strict phenylalanine control [13]. Whether a similar gradient exists at the population level across the much larger group of infants with borderline phenylalanine elevation at screening, who do not receive dietary intervention, has rarely been examined using unselected, population-based data; size at birth, measured before any dietary treatment, offers a way to examine this without confounding by treatment; prior characterizations of screen-positive infants have generally been clinic- or referral-based case series rather than population-based analyses [14].

The borderline population therefore raises a different epidemiological question from classical PKU: rather than case ascertainment for a rare disease, it asks what distinguishes children with a mildly elevated screening phenylalanine level, and whether this large, untreated group has any detectable downstream correlate. The present analysis uses a large regional maternal-child health registry of 543,952 children with a valid newborn screening phenylalanine value to address three aims: (1) to describe the distribution of borderline and treatment-range newborn phenylalanine and compare treatment-range prevalence with published Iranian and international estimates; (2) to identify clinical and sociodemographic predictors of borderline phenylalanine status, including parental consanguinity; and (3) to examine whether borderline and treatment-range phenylalanine are associated with weight and length at birth, before any dietary treatment.

Methods

Data source and study population

Data were drawn from Sina, a regional maternal-child health information system covering the full population served by Mashhad University of Medical Sciences (MUMS), northeastern Iran, distinct from but periodically synchronized with Iran's national Integrated Health System (Sib). The MUMS catchment population is approximately 5.5 million, officially reported as 80% of Razavi Khorasan province's 6.87 million residents, across 17 counties. This analysis is a complete census of all births recorded in this network between the Iranian solar years 1396 and 1403 (approximately 2017–2025 Gregorian), comprising 548,318 children, of whom 543,956 (99.2%) had a recorded newborn phenylalanine value; births in 1396 and 1403 were only partly covered.

Phenylalanine ascertainment and categorization

Newborn phenylalanine concentration (mg/dL) was measured in dried heel-prick blood spots collected at 3–5 days of age, the same collection window used for newborn TSH screening, as part of Iran's national newborn screening program, which includes mandatory PKU screening [15, 16]. The registry records the result of the initial screening test for each child; results of recall and repeat testing and final diagnoses are not recorded. The registry stores the quantitative phenylalanine value only; the assay method, screening laboratory and recall cut-off are not recorded. Four records with implausible negative values were treated as missing. Phenylalanine was categorized using internationally accepted thresholds [1, 4, 7]: normal (< 2 mg/dL; <120 µmol/L, the upper limit of normal); borderline (2 to < 6 mg/dL; 120 to < 360 µmol/L), a range that on repeat testing may correspond to mild hyperphenylalaninemia but in most infants reflects transient or false-positive elevation; and treatment-range hyperphenylalaninemia (≥ 6 mg/dL; ≥360 µmol/L), the level at which dietary treatment is generally recommended. Because the registry records a single screening value per child and no confirmatory, diagnostic or treatment data, this analysis cannot distinguish persistent from transient elevation or identify which treatment-range children had PKU.

Covariates

Covariates were selected a priori as plausible determinants of newborn phenylalanine or of size at birth that precede the newborn screen. The predictor model (Table 2) and the birth-size models included maternal age, pre-pregnancy BMI, parity, maternal education (registry ordinal scale, 0–10), parental consanguinity (in the predictor model), child sex, preterm birth, multiple birth, gestational diabetes and maternal anemia. Preterm birth was taken from the registry's binary preterm-birth indicator; gestational age in weeks was not recorded and could not be used. Maternal ages outside 12–55 years were treated as missing. Birth year (Iranian solar year) was included in all models as a categorical variable. Continuous predictors were standardized using SDs of 6.7 years (maternal age), 4.9 kg/m² (BMI), 1.4 pregnancies (parity) and 2.1 levels (education).

Birth size outcomes

Weight-for-age and length-for-age z-scores at birth were computed from recorded birth weight and length using the sex-specific WHO Child Growth Standards (2006) LMS values at birth [17]; values beyond ± 6 SD were treated as implausible and excluded.

Statistical analysis

The prevalence of borderline and treatment-range phenylalanine was described overall and by birth year, and compared narratively with published Iranian and international estimates. Because the proportion of borderline results varied markedly across birth years (Fig. 1), all regression models were adjusted for birth year as a categorical variable. To assess whether results depended on the fixed 2 mg/dL cut-off in the presence of year-to-year laboratory variation, predictors were re-estimated using (i) a year-specific definition (phenylalanine above the 90th percentile of its birth year), (ii) phenylalanine standardized within birth year as a continuous outcome (linear regression), and (iii) the fixed cut-off restricted to 1396–1397, the years with the lowest and most stable proportion of borderline results. Measurement drift was assessed by comparing percentiles of the phenylalanine distribution across birth years, comparing them with newborn TSH measured in the same dried blood spot, testing whether the year-to-year shift differed between subgroups (residence type, sex, preterm birth), and estimating how much of the shift was explained by child and maternal characteristics. Values were then harmonized to the 1396–1397 distribution, the reference period in which the 99th percentile (2.0 mg/dL) matched the conventional upper limit of normal, by (iv) quantile mapping, in which each value was replaced by the 1396–1397 value at the same within-year percentile rank, and (v) subtracting the difference between each year's median and the reference median. Children were reclassified at 2 mg/dL and the predictor and consanguinity analyses were repeated; treatment-range classification was based on unharmonized values. Predictors of borderline status were identified using multivariable logistic regression (borderline vs normal; treatment-range children were excluded because of their small number and distinct etiology), with maternal age, pre-pregnancy BMI, parity, maternal education, child sex, preterm birth, multiple birth,

gestational diabetes, maternal anemia and parental consanguinity as predictors; continuous predictors were standardized. Residence type (five categories) was added in a further sensitivity analysis, because screening laboratories and several predictors vary geographically. The main analyses used complete cases; in sensitivity analyses, pre-pregnancy BMI (the variable with most missing data) was omitted, and missing covariates were imputed by chained equations (10 imputations including all model variables, the outcome and birth year), with estimates combined using Rubin's rules. The registry's consanguinity grading changed during the study period: Grades 1 (first cousins) and 2 were used only from 1400 onward, and before 1400 a larger share of consanguineous marriages was coded as Grade 3, whereas the proportion with any consanguinity was stable across years (Additional file 1, Table S3). Consanguinity was therefore analyzed as any versus none, and grade-specific associations were examined only for births in 1400–1403 (Additional file 1, Table S6). The association between treatment-range phenylalanine and consanguinity was estimated with a Mantel–Haenszel odds ratio stratified by birth year, and a multinomial logistic model (normal, borderline, treatment-range) including consanguinity and birth year was used to test whether the consanguinity association differed between the borderline and treatment-range categories (Wald test). Because the treatment-range group may include infants with secondary elevation related to prematurity or neonatal illness, this association was also estimated after excluding preterm infants, multiple births and infants hospitalized after birth, and after restricting cases to values ≥ 10 mg/dL; exact unadjusted odds ratios are reported alongside Mantel–Haenszel estimates. Weight-for-age and length-for-age z-scores at birth were regressed on phenylalanine category with the same covariates and birth year, using heteroskedasticity-robust standard errors, in all children and in term singletons. Benjamini–Hochberg false discovery rate (FDR) correction was applied within the predictors-of-borderline model, and FDR-corrected p-values (p_FDR) are reported alongside raw p-values. Statistical significance was set at $\alpha = 0.05$ (two-sided). This analysis was exploratory: neither the prevalence estimates nor the birth-size associations were pre-specified hypotheses, and the consanguinity analysis was added post hoc. Reporting follows the STROBE statement and its RECORD extension for routinely collected health data (Additional file 2).

Software

All data processing and statistical analysis were conducted in Python 3.12.3, using pandas, NumPy and SciPy for data management and statistical computation, statsmodels (version 0.15.0) for regression modeling, and openpyxl and lxml for source-file extraction and parsing. WHO reference tables were obtained directly from who.int. The analysis code is provided in Additional file 3. No causal inference methods were applied, as no maternal, family, or sibling linkage identifier was available in the source data; all reported associations are therefore adjusted observational associations.

Results

Prevalence of newborn phenylalanine elevation

Of 543,956 children with a recorded newborn phenylalanine value, four had implausible negative values, leaving 543,952 for analysis. Of these, 57,402 (10.5%) had a borderline value (2 to < 6 mg/dL; 120 to < 360 µmol/L) and 63 (1 in 8,634; 11.6 per 100,000) had a treatment-range value (≥ 6 mg/dL; ≥360 µmol/L) (Table 1; Additional file 1, Figure S1). The proportion with a borderline value varied markedly by birth year, from 1.1% in 1396 and 1397 to 25.5% in 1402, with an upward shift in median phenylalanine from 1.2 to 1.7 mg/dL, whereas the number of treatment-range cases per year was stable (Fig. 1; Additional file 1, Table S3). Characteristics of children by phenylalanine category are shown in Additional file 1, Table S1. The upward shift affected the whole distribution in parallel, from the 5th to the 99th percentile, and was the same in every residence type, in boys and girls, and in preterm and term infants, whereas the median newborn TSH measured in the same blood spot did not change (Additional file 1, Figure S2 and Table S8). Child and maternal characteristics explained none of the year-to-year shift (adjustment changed the year effects by less than 1.5%), and birth year explained 13.8% of the variance in phenylalanine, compared with 1.2% for all characteristics combined. After harmonization to the 1396–1397 distribution, about 1% of children had a borderline value in every year (overall 1.1%, n = 5,742) by quantile mapping, and 4.5% by median-offset correction.

Table 1
Distribution of newborn screening phenylalanine category (N = 543,952). Proportions are not corrected for measurement drift between birth years (see Fig. 1 and Additional file 1, Tables S3 and S8).

Category	n	Prevalence
Normal (< 2 mg/dL; <120 µmol/L)	486,487	89.4%
Borderline (2 to < 6 mg/dL; 120 to < 360 µmol/L)	57,402	10.5% (1.1–25.5% by birth year)
Treatment-range (≥ 6 mg/dL; ≥360 µmol/L)	63	0.012% (1 in 8,634)

Predictors of borderline phenylalanine status

The multivariable logistic regression included 394,648 children with complete data on all predictors; exclusions were mainly due to missing pre-pregnancy BMI (Additional file 1, Figure S1). Excluded children were more often urban residents and slightly more often had a borderline value (11.3% vs 10.3%) (Additional file 1, Table S2). After adjustment for birth year, borderline status was positively associated with gestational diabetes (OR 1.27, 95% CI 1.13–1.43), preterm birth (OR 1.20, 1.15–1.26), multiple birth (OR 1.10, 1.03–1.17), female sex (OR 1.09, 1.07–1.12), maternal education (OR 1.04 per SD, 1.03–1.05) and maternal age (OR 1.03 per SD, 1.02–1.04) (Table 2). Adding residence type did not change any estimate by more than 0.01. Estimates were almost identical when BMI was omitted (N = 507,502) and with multiple imputation (N = 543,889) (Additional file 1, Table S10). It was not associated with parental consanguinity (OR 1.00, 0.98–1.03), maternal BMI, parity or maternal anemia. Without adjustment for birth year, consanguinity, maternal age, parity, BMI and anemia all appeared to be associated with borderline status (Additional file 1, Table S4), indicating that these apparent associations reflected differences between birth years. Among births in 1400–1403, when all consanguinity grades were in use, no grade was positively associated with borderline status (Additional

file 1, Table S6). In sensitivity analyses (Additional file 1, Table S7), preterm birth was associated with higher phenylalanine under every definition, including in 1396–1397 alone (OR 1.89, 95% CI 1.45–2.46), and gestational diabetes and female sex were associated under all definitions covering the whole study period. After quantile harmonization, preterm birth (OR 1.53, 95% CI 1.35–1.74) and female sex (OR 1.09, 1.03–1.16) remained associated with borderline status, whereas the association with gestational diabetes was no longer statistically significant (OR 1.07, 0.75–1.52). Associations with maternal education and maternal age were small and inconsistent across definitions. Parental consanguinity showed at most a weak association under any definition (year-specific definition: OR 1.03, 1.00–1.05; 1396–1397 only: OR 1.10, 0.96–1.26; quantile harmonization: OR 1.00, 0.94–1.08), well below the association observed for treatment-range phenylalanine.

Table 2
Multivariable logistic regression: predictors of borderline phenylalanine status (2 to < 6 mg/dL vs normal, < 2 mg/dL), adjusted for birth year, N = 394,648.

Predictor	OR (95% CI)	p	p_FDR
Any parental consanguinity	1.00 (0.98–1.03)	0.821	0.954
Maternal age (per SD)	1.03 (1.02–1.04)	< 0.001	< 0.001
Maternal BMI (per SD)	1.00 (0.99–1.01)	0.984	0.984
Parity (per SD)	1.00 (0.99–1.01)	0.858	0.954
Female sex	1.09 (1.07–1.12)	< 0.001	< 0.001
Preterm birth	1.20 (1.15–1.26)	< 0.001	< 0.001
Multiple birth	1.10 (1.03–1.17)	0.005	0.008
Gestational diabetes	1.27 (1.13–1.43)	< 0.001	< 0.001
Maternal anemia	0.94 (0.79–1.13)	0.517	0.739
Maternal education (per SD)	1.04 (1.03–1.05)	< 0.001	< 0.001

Phenylalanine category and size at birth

After adjustment for covariates and birth year, borderline phenylalanine was associated with slightly lower weight-for-age (β − 0.07, 95% CI − 0.08 to − 0.06) and length-for-age (β − 0.06, − 0.07 to − 0.05) z-scores at birth, and the differences were similar in term singletons (− 0.06 and − 0.05). Treatment-range phenylalanine was associated with a lower weight-for-age z-score at birth (β − 0.47, − 0.78 to − 0.17; term singletons − 0.39, − 0.68 to − 0.11) but not with length-for-age (β − 0.01, − 0.33 to 0.31) (Additional file 1, Table S5).

Association with parental consanguinity

Because PKU is autosomal recessive, a post hoc analysis examined whether treatment-range phenylalanine was associated with parental consanguinity, using the registry's five-level classification (non-consanguineous; Grade 1, first cousins; Grades 2, 3 and 4 or more, progressively more distant relationships), which was available for 97.5% of children. Because the grading changed in 1400, the primary comparison was any consanguinity versus none.

Table 3
Treatment-range phenylalanine (≥ 6 mg/dL) by parental consanguinity grade, all birth years (530,542 children with known consanguinity; 60 cases). Grades 1 and 2 were used only from 1400 onward, so grade-specific rates are not comparable across the study period (Additional file 1, Table S3).

Consanguinity grade	N	Treatment-range cases, n (per 100,000)
Non-consanguineous (reference)	383,493	27 (7.0)
Grade 1 (first cousin)	27,540	8 (29.0)
Grade 2	8,712	0 (0.0)
Grade 3	69,334	22 (31.7)
Grade 4 or more	41,463	3 (7.2)

Treatment-range phenylalanine was more common among children of consanguineous parents than among children of non-consanguineous parents (33 of 147,049 [22.4 per 100,000] vs 27 of 383,493 [7.0 per 100,000]; absolute difference 15.4 per 100,000; Mantel–Haenszel OR stratified by birth year 3.18, 95% CI 1.91–5.28; $p < 0.001$). In the multinomial model adjusted for birth year, any consanguinity was associated with treatment-range phenylalanine (OR 3.19, 95% CI 1.92–5.30) but not with borderline phenylalanine (OR 0.99, 0.97–1.01), and the two associations differed significantly (Wald $z = 4.51$, $p < 0.001$). The difference persisted after quantile harmonization (borderline OR 1.02, 0.96–1.08; treatment-range OR 3.19, 1.92–5.31; $p < 0.001$) and after median-offset correction (Additional file 1, Table S7). Treatment-range children were more often preterm (17.5% vs 5.1%) and hospitalized after birth (28.6% vs 9.4%) than children with normal values. The association with consanguinity persisted after excluding preterm infants, multiple births and infants hospitalized after birth (Mantel–Haenszel OR 3.40, 95% CI 1.85–6.26; 42 cases), was similar with exact estimation, and was weaker and imprecise when cases were restricted to values ≥ 10 mg/dL (OR 2.05, 0.93–4.52; 25 cases) (Additional file 1, Table S9). By grade, treatment-range cases were most frequent in Grade 1 (first cousins; 29.0 per 100,000) and Grade 3 (31.7 per 100,000) (Table 3), but grade-specific estimates are imprecise because of the small number of cases (33 among consanguineous families) and are affected by the change in grading in 1400.

Discussion

Summary of findings

In this regional registry of 543,952 children with a newborn screening phenylalanine value, borderline phenylalanine (10.5%) was far more common than treatment-range hyperphenylalaninemia (11.6 per 100,000), but its frequency varied more than 20-fold between birth years because of measurement drift, and after harmonization about 1% of children had borderline values. After accounting for birth year, borderline status had modest perinatal predictors (most consistently preterm birth, gestational diabetes and female sex) and was not associated with parental consanguinity under any definition, whereas treatment-range hyperphenylalaninemia was about three times more common among children of consanguineous parents. Both categories were associated with smaller size at birth, and for the borderline group this persisted in term singletons.

Interpretation

The association between consanguinity and treatment-range hyperphenylalaninemia is what autosomal recessive inheritance predicts [18]: consanguineous parents are more likely to both carry, and jointly transmit, the same phenylalanine hydroxylase variant. The treatment-range group probably also includes infants with secondary hyperphenylalaninemia, as suggested by the higher proportions of preterm and hospitalized newborns, but the association with consanguinity was, if anything, stronger after these infants were excluded, which supports a genetic contribution. Borderline phenylalanine, by contrast, showed no association with consanguinity once birth year was taken into account, and the two associations differed significantly. Because year-to-year laboratory variation could have diluted a true association through misclassification, the analysis was repeated with year-specific definitions and restricted to the most stable years; the association with consanguinity remained weak in every case, with upper confidence limits no higher than 1.26, far below the odds ratio of about 3 for treatment-range phenylalanine. This supports the view that the borderline and treatment-range groups are largely distinct populations rather than points on a single continuum of phenylalanine hydroxylase impairment, and that findings from diagnosed cases should not be assumed to apply to the much larger borderline group. It also shows why adjustment for birth year was necessary: without it, consanguinity appeared to be inversely associated with borderline status, because the consanguinity grading and the proportion of borderline results both changed over the same period.

The more than 20-fold variation in the proportion of borderline results between birth years reflects a measurement change rather than a change in the population. The whole phenylalanine distribution shifted in parallel, the shift was identical across subgroups and was not explained by child or maternal characteristics, newborn TSH from the same blood spot did not shift, and the number of treatment-range cases was stable. Information on changes in assay, laboratory or calibration was not available in the registry. The unadjusted figure of 10.5% therefore greatly overstates the proportion of children with elevated phenylalanine at screening; after harmonization to the earliest years, when the 99th percentile was 2.0 mg/dL, the proportion was about 1%. Because a fixed cut-off applied to drifting values produces misclassification that can create or hide associations, analyses of borderline status in routine screening data need to account for calibration over time, and screening programs should monitor population percentiles as a routine quality indicator.

This drift has direct consequences for families. The registry does not record recalls and the program's recall cut-off is not documented, but if recall decisions were based on a fixed threshold within the range affected by the drift, the number of infants called back for repeat testing would have risen in step with the proportion of borderline results, from about 1 in 90 births in 1396–1397 to about 1 in 4 in 1402, without any change in the number of affected infants. Each recall means a further blood sample and a return visit in the first weeks of life, and false-positive newborn screening results are a recognized source of parental anxiety and additional health-service use [19]. Recall volumes of this size would also strain follow-up capacity and could delay the evaluation of the few infants with true phenylketonuria. Routine monitoring of population percentiles and recall rates by laboratory and year, with prompt recalibration when they shift, would protect families from unnecessary recalls at little cost.

The treatment-range prevalence observed here (11.6 per 100,000) is based on a single screening value ≥ 6 mg/dL rather than on confirmed PKU diagnoses, so it is not directly comparable with estimates of diagnosed PKU. With that caveat, it is somewhat lower than the pooled Iranian national estimate for PKU (16.5 per 100,000) [9], falls within the global pooled birth-prevalence range reported by a recent systematic review and meta-analysis of phenylalanine hydroxylase deficiency [20], and sits within the substantial heterogeneity previously documented across Iranian provinces, which has been attributed in part to regional variation in consanguineous marriage rates given PKU's autosomal recessive inheritance [8, 10]. Accurate classification matters because even mild phenylalanine elevation has been linked to neurodevelopmental risk [21], and delayed recognition of true PKU has lasting consequences [22]. The lower size at birth in the treatment-range group cannot reflect dietary treatment, which begins after screening; it may instead reflect confounding by prematurity or neonatal illness, both of which also raise phenylalanine [23, 24]. Studies of children with treated PKU have reported reduced growth, particularly with poorer metabolic control [11, 12, 25], but because this registry has no serial measurements or treatment data, we cannot determine how many children in the treatment-range group were treated, nor distinguish benign hyperphenylalaninemia from treated mild or classical PKU.

The association of borderline phenylalanine with slightly smaller size at birth (about 0.06–0.07 SD) has not, to our knowledge, been reported at this scale. Newborn screening programs generally identify many infants with transient abnormalities that resolve on follow-up [19], and the small effect observed here should not be over-interpreted clinically. It may reflect residual confounding by factors associated with both transient neonatal phenylalanine elevation and birth size, such as prematurity-related metabolic immaturity [23], acute neonatal illness or parenteral nutrition [24], or the timing of blood collection relative to birth and the normal postnatal phenylalanine wash-out pattern [26, 27]. Because gestational age was unavailable, birth-size z-scores could not be corrected for gestational age, and preterm infants, who are more likely to have elevated phenylalanine, will have lower uncorrected z-scores. However, the borderline difference persisted in term singletons after adjustment for birth year (Additional file 1, Table S5), so prematurity is unlikely to explain it entirely. The small but consistent excess of borderline values among girls has not, to our knowledge, been well described and warrants replication.

Clinical versus statistical significance

Given the very large sample, statistical significance was reached for effects that are small in absolute and clinical terms. The borderline-category differences in size at birth (about − 0.06 to − 0.07 SD) are small relative to the 2 SD threshold conventionally used to define growth faltering, and the ORs for most predictors of borderline status are modest. These estimates should be interpreted as epidemiological signals rather than as evidence of clinically actionable individual-level risk. The treatment-range estimates are larger but imprecise, because they are based on only 63 children.

Strengths

This analysis draws on a very large, population-scale registry with near-complete newborn phenylalanine ascertainment (99.2%), allowing precise estimation of the proportion of children with borderline phenylalanine. Size at birth was measured before any dietary treatment, expressed as WHO-standard, sex-specific z-scores, and examined in term singletons as well as in all children.

Limitations

Several limitations should inform interpretation. First, county, health center and screening laboratory were not recorded, so clustering at these levels could not be modeled, although adjustment for residence type did not change the estimates; no reliable maternal identifier was available, so siblings could not be accounted for and no within-family comparison was possible. Second, the registry records only the initial screening value, without repeat measurements, confirmatory diagnoses or treatment data, so persistent and transient elevation cannot be distinguished. Third, the proportion of children with a borderline value varied from 1.1% to 25.5% between birth years, which the internal evidence indicates was a measurement change rather than a population change; all models were adjusted for birth year and the main findings were similar with year-specific definitions, but differences between laboratories within a year could not be examined because the screening laboratory was not recorded. Parental consanguinity grading also changed in 1400, so only any versus no consanguinity could be compared across the whole period. Fourth, gestational age was unavailable, so birth-size z-scores could not be corrected for gestational age, and prematurity could be adjusted for only as a binary variable. Fifth, the treatment-range group was small (n = 63), so estimates for this group are imprecise, and these children could not be linked to clinical PKU diagnoses; the group is therefore likely to combine true phenylalanine hydroxylase deficiency with secondary elevation. Sixth, the main analyses used complete cases, and excluded children, who were mostly missing pre-pregnancy BMI, were more often urban residents (Additional file 1, Table S2); however, estimates were almost unchanged when BMI was omitted and with multiple imputation. Seventh, this is a census of the MUMS-covered population in northeastern Iran, not a national sample; consanguinity rates and PKU prevalence vary across Iranian regions, and findings should not be generalized without further validation. Finally, the analysis was exploratory and not pre-registered; findings should be regarded as hypothesis-generating pending independent replication.

Future directions

Linking this registry's phenylalanine data to dietary-management records would allow the treatment-range group to be separated into benign hyperphenylalaninemia and treated PKU, clarifying the clinical

significance of treatment-range values. Serial phenylalanine measurements would allow the borderline category to be divided into persistent and transient elevation. Linkage to gestational age and neonatal intensive care records would allow confounding by prematurity and neonatal illness to be addressed directly, and laboratory records would allow year-specific calibration to be modeled explicitly. Growth after the newborn period was not examined here and should be analyzed with methods that account for measurement drift in the exposure.

Conclusions

Borderline phenylalanine at newborn screening was common in this population, but most of the apparent borderline population reflected measurement drift between birth years; after harmonization, about 1% of children had borderline values. After accounting for birth year, it had modest perinatal predictors, was not associated with parental consanguinity, and was associated with slightly smaller size at birth. Treatment-range hyperphenylalaninemia, in contrast, was strongly associated with parental consanguinity, as expected for an autosomal recessive condition. These findings are exploratory and should motivate replication in registries with serial phenylalanine measurements, gestational age, laboratory and treatment data.

Abbreviations

BMI
Body mass index
CI
Confidence interval
FDR
False discovery rate
LMS
Lambda-mu-sigma
MUMS
Mashhad University of Medical Sciences
OR
Odds ratio
PKU
Phenylketonuria
RECORD
REporting of studies Conducted using Observational Routinely-collected health Data
SD
Standard deviation
STROBE
Strengthening the Reporting of Observational Studies in Epidemiology
TSH

Thyroid-stimulating hormone
WHO
World Health Organization.

Declarations

Ethics Approval and Consent to Participate

This study used de-identified secondary data from an existing regional maternal-child health registry covering the population served by Mashhad University of Medical Sciences (MUMS). The study protocol was approved by the MUMS Research Ethics Committee (approval code: IR.MUMS.MEDICAL.REC.1404.189). All methods were carried out in accordance with relevant guidelines and regulations and with the ethical principles of the Declaration of Helsinki.

All participants were newborns and young children (minors). Because the study was a retrospective analysis of routinely collected, de-identified registry data, with no contact with participants and no access by the investigator to personal identifiers, the MUMS Research Ethics Committee waived the requirement for informed consent, including informed consent from the participants' parents or legal guardians.

Consent for Publication

Not applicable.

Availability of Data and Materials

The dataset analyzed in this study was derived from a regional maternal-child health registry maintained by Mashhad University of Medical Sciences (MUMS). The data are not publicly available due to institutional data-governance restrictions protecting patient confidentiality, but are available from the corresponding author upon reasonable request and subject to approval by the registry's data custodian and, where applicable, the MUMS Research Ethics Committee. The analysis code is provided in Additional file 3.

Competing Interests

The author declares no competing interests.

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Authors' Contributions

Veda Vakili: Conceptualization, Methodology, Formal analysis, Data curation, Investigation, Writing – original draft, Writing – review & editing, Supervision.

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Figures

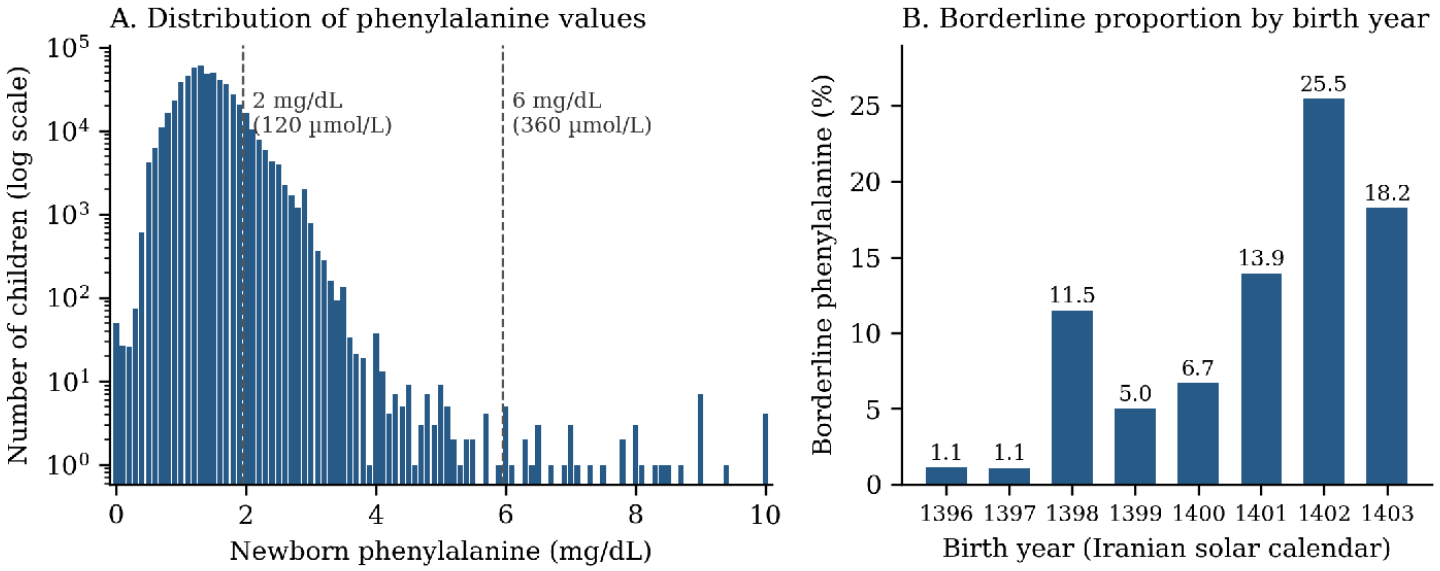


Figure 1

Newborn phenylalanine distribution and borderline proportion by birth year. (A) Distribution of newborn screening phenylalanine values between 0 and 10 mg/dL (21 values above 10 mg/dL not shown), log scale; dashed lines mark 2 mg/dL (120 µmol/L) and 6 mg/dL (360 µmol/L). (B) Percentage of children with borderline phenylalanine (2 to <6 mg/dL) by birth year (Iranian solar calendar; 1396 ≈ 2017–18, 1403 ≈ 2024–25).

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