

Supplementary Table S1. Regional distribution of ages at admission, DBS collection, and phenylalanine result times. Data presented in days, as median (Q1-Q3), min-max.

Region	Age at admission	Age at 1st DBS collection	Age at 2nd DBS collection	Age at 3rd DBS collection	Time of 1st DBS Phe result	Time of 2nd DBS Phe result	Time of 3rd DBS Phe result
Central Anatolia 56.4% (n=622)	23 (17-32), 3-302	1 (1-2), 0-61	6 (5-10), 3-178	15 (13-20), 7-78	5 (4-7), 1-40	6 (4-8), 1-34	6 (4-9), 1-37
Black Sea 27.6% (n=304)	26 (19-33), 6-313	3 (1-6), 0-17	11 (6-16), 3-64	17 (14-22.5), 10-73	7 (5-10), 2-62	7 (5-9), 2-41	7 (5-8.5), 2-30
Mediterranean 1.0% (n=11)	40 (26-174), 8-410	2 (1-5), 1-49	20 (7-45.5), 3-157	51.5 (29-74), 29-74	10 (7-14), 4-17	7.5 (7-12.5), 3-27	10 (5-15), 5-15
Marmara 0.7% (n=8)	19.5 (16.5-28), 11-261	1 (1-1), 0-3	6 (6-7), 5-10	16 (13-118), 11-219	5 (4-7.5), 2-10	6 (5-9), 4-10	6.5 (3-15), 2-21
Aegean 1.4% (n=15)	24 (15-28), 7-138	4 (2-8), 1-13	10 (7-17), 6-20	13 (10-31), 8-34	4 (3-5), 1-7	4 (4-7), 2-7	6 (5-11), 4-11
Southeastern Anatolia 2.3% (n=25)	34 (27-42), 11-152	1 (0-3), 0-14	8 (6-17), 4-90	29 (19-47), 15-103	8 (6.5-10), 2-45	8 (6-11), 3-32	7 (6-11), 5-16
Eastern Anatolia 10.7% (n=118)	34 (25-42), 11-224	1 (0-4), 0-734	7 (5-14), 3-54	18 (15-31), 10-60	8 (6-11), 3-30	8 (6-12), 3-42	7 (5-12), 2-41
Overall	20.5 (17.5-25.5), 12-62	1 (1-4), 0-734	7 (5-13), 3-178	16 (13-21), 16-219	6 (4-8), 1-62	7 (5-9), 1-42	7 (5-9), 1-41

*This table presents the regional distribution of admission dates, dried blood spot (DBS) collection times, and phenylalanine (Phe) result times across different geographic regions. Data is expressed as median (Q1-Q3) and min-max values (days). Age at admission date refers to the time from birth to hospital admission. DBS collection times (1st, 2nd, and 3rd) indicate the age (in days) when blood samples were collected. Phe result times represent the time taken for each DBS Phe result to be available. In the Black Sea region, the first dry blood spot (DBS) sampling was performed at a later time, while the second and third DBS sampling was performed at older ages. In the Southeastern Anatolia region, the timing of the first DBS sampling was similar to the other regions, but there was a significant delay in the second and third DBS sampling. In addition, the interval between DBS measurements is longer in this region.

Supplementary Table S2. *P* values of regional comparisons of DBS sample collection and result processing times.

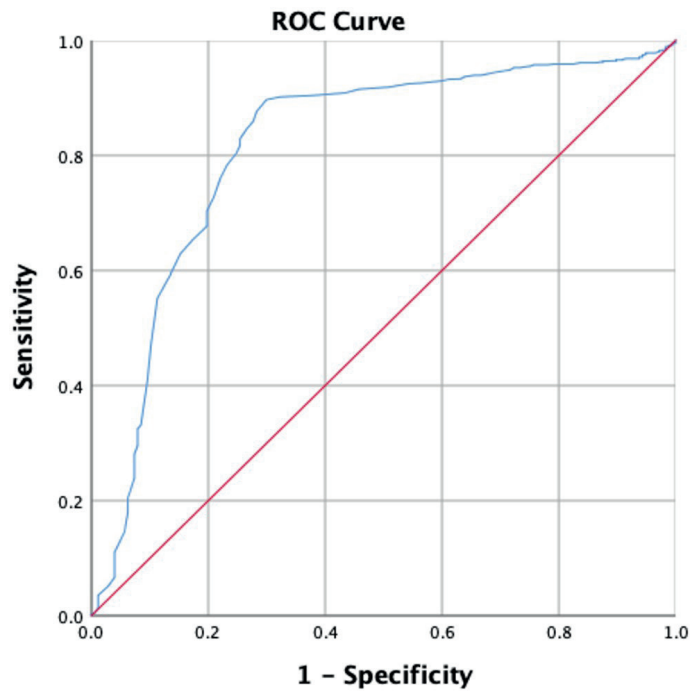
Region	Age at admission	Age at 1st DBS collection	Age at 2nd DBS collection	Age at 3rd DBS collection	Time of 1st DBS result	Time of 2nd DBS result
1 vs. 2	0.1	<0.001	<0.001	0.001	<0.001	<0.001
1 vs. 3	0.01	0.02	0.01	0.02	0.002	0.09
1 vs. 4	0.44	0.41	0.88	0.59	0.89	0.79
1 vs. 5	0.6	<0.001	0.01	0.47	0.02	0.08
1 vs. 6	<0.001	0.09	0.03	<0.001	<0.001	0.036
1 vs. 7	<0.001	0.98	0.09	<0.001	<0.001	<0.001
2 vs. 3	0.02	0.68	0.12	0.04	0.12	0.34
2 vs. 4	0.3	0.006	0.02	0.84	0.07	0.53
2 vs. 5	0.31	0.17	0.73	0.17	<0.001	0.011
2 vs. 6	0.001	0.001	0.62	0.01	0.22	0.39
2 vs. 7	<0.001	<0.001	0.001	0.41	0.014	0.005
3 vs. 4	0.1	0.07	0.07	0.53	0.03	0.19
3 vs. 5	0.03	0.25	0.27	0.28	<0.001	0.021
3 vs. 6	0.52	0.051	0.23	0.41	0.44	0.88
3 vs. 7	0.3	0.1	0.053	0.089	0.44	0.96
4 vs. 5	0.97	0.003	0.02	0.39	0.14	0.17
4 vs. 6	0.02	0.72	0.13	0.41	0.04	0.26
4 vs. 7	0.02	0.55	0.51	0.77	0.01	0.09
5 vs. 6	0.01	0.004	0.6	0.04	<0.001	0.01
5 vs. 7	0.008	0.002	0.16	0.15	<0.001	0.001
6 vs. 7	0.63	0.25	0.27	0.04	0.93	0.66

*This table illustrates the differences in the timing of DBS sample collection and result processing among various regions. The significance threshold was set at $p < 0.0167$ after applying the Bonferroni correction, and *p* values < 0.0167 are printed bold. The regions; (1) Central Anatolia, (2) Black Sea, (3) Mediterranean, (4) Marmara, (5) Aegean, (6) Southeastern Anatolia, (7) Eastern Anatolia. Significant differences in DBS sampling timelines and result processing durations are observed between the Central Anatolia region and the Black Sea, Southeastern Anatolia, and Eastern Anatolia regions. For descriptive values, please refer to Supplementary Table S1.

Supplementary Table S3. Phenylalanine levels across different clinical and neonatal conditions. Data presented in mg/dL as median (Q1-Q3), min-max.

	1st DBS Phe	2nd DBS Phe	3rd DBS Phe	1st Plasma Phe
Neonatal Hospitalization (+) (n=182)	2.65 (2.1-3.9), 0.5-29.2	2.4 (2.2-3.2), 0.6-16.8	2.5 (2.3-2.9), 1.3-6.6	2.1 (1.2-3.4), 0.1-55
Neonatal Hospitalization (-) (n=921)	2.5 (1.6-3.6), 0.2-40	2.5 (2.2-3.2), 0.4-32	2.4 (2.2-2.7), 0.6-5.6	2.2 (1.2-3.1), 0.5-47
p	0.02	0.76	0.03	0.9
LBW (+) (n=92)	2.5 (2.1-3.8), 0.5-29.2	2.4 (2.1-3.15), 0.6-11	2.3 (2.1-2.5), 1.6-5.2	1.62 (1.1-2.9), 0.5-47
LBW (-) (n=965)	2.5 (1.6-3.7), 0.2-40	2.5 (2.2-3.3), 0.7-32	2.4 (2.2-2.8), 0.6-6.6	2.2 (1.3-3.4), 0.5-55
p	0.69	0.19	0.12	0.01
Antibiotic use (+) (n=71)	2.5 (1.7-3.8), 0.7-16.3	2.7 (2.3-3.8), 2-16.4	2.45 (2.2-2.8), 2-4.3	2.19 (1.2-3.1), 0.6-37
Antibiotic use (-) (n=1032)	2.5 (1.7-3.7), 0.2-40	2.5 (2.2-3.2), 0.4-32	2.4 (2.2-3.7), 0.6-6.6	2.1 (1.26-3.3), 0.1-55
p	0.87	0.06	0.37	0.79
Gestational age at birth <37w (n=142)	2.5 (1.95-3.9), 0.5-25	2.3 (2.1-2.8), 0.6-16.8	2.3(2.1-2.7), 1.3-4.1	1.7 (1-2.9), 0.1-35
Gestational age at birth ≥37w (n=961)	2.5 (1.6-3.7), 0.2-40	2.5 (2.2-3.3), 0.4-32	2.4 (2.2-2.7), 0.6-6.6	2.1 (1.3-3.4), 0.5-55
p	0.68	<0.001	0.39	<0.001
Neonatal jaundice (+) (n=167)	2.7 (2.1-4.1), 0.5-29.3	2.5 (2.2-3.3), 0.6-16.8	2.5 (2.2-2.8), 1.3-6.6	2.35 (1.3-3.3), 0.6-47
Neonatal jaundice (-) (n=936)	2.5 (1.6-3.6), 0.2-40	2.5 (2.2-3.2), 0.4-32	2.4 (2.2-2.7), 0.6-5.6	2 (1.2-3.3), 0.1-55
p	0.02	0.35	0.05	0.06
Phototherapy (+) (n=81)	2.7 (2.2-3.9), 0.5-29.2	2.4 (2.3-3.2), 0.6-16.8	2.6 (2.2-3.0), 1.3-6.6	2.3 (1.3-3.0), 0.6-47
Phototherapy (-) (n=86)	2.7 (1.9-4.1), 0.5-25	2.6 (2.2-3.5), 0.8-11	2.5 (2.2-2.8), 1.6-4.4	2.4 (1.5-4.3), 0.8-43
p	0.47	0.73	0.27	0.14
Group 1; Phe 2–6 mg/dL (n=422)	3 (2.3-3.7), 0.5-7.3	2.9 (2.5-3.7), 1.1-8	2.7 (2.4-3.2), 1.1-6.6	2.9 (2.3-3.6), 0.7-9.6
Normal (n=421)	1.8 (1.1-2.4), 0.2-20	2.2 (2.1-2.4), 0.4-9.9	2.3 (2.1-2.5), 0.6-5.2	1.2 (0.9-1.5), 0.1-2
p	<0.001	<0.001	<0.001	<0.001
Maternal PKU (n=5)	9.3 (7.9-11.7), 4.9-24.5	N/A	N/A	1.21 (1.09-1.37), 0.8-1.4
Transient HPA (n=26)	2.3 (1.7-3.1), 0.2-6	N/A	N/A	2.1 (1.7-2.3), 0.6-5.6
p	<0.001	N/A	N/A	0.003
Transient HPA/tyrosinemia (n=98)	2.1 (1.4-2.6), 0.2-11.7	2.3 (2.1-2.6), 0.6-6.8	2.35 (2.2-2.6), 1.6-4.3	1.69 (1.2-2.3), 0.6-6.7
Normal (n=421)	1.8 (1.1-2.4), 0.2-20	2.2 (2.1-2.4), 0.4-9.9	2.3 (2.1-2.5), 0.6-5.2	1.2 (0.9-1.5), 0.1-2
p	0.01	0.29	0.055	<0.001

P<0.05 written in bold. This table presents the 1st, 2nd, and 3rd dried blood spot (DBS) phenylalanine (Phe) levels from the newborn screening program and 1st plasma Phe levels measured at our metabolic clinic across various neonatal and clinical conditions. Median values with interquartile ranges and minimum-maximum values are reported for each group. Neonatal hospitalization, low birth weight (LBW), antibiotic use, gestational age (<37 weeks), neonatal jaundice, and phototherapy, as well as specific subgroups including maternal PKU, transient hyperphenylalaninemia (HPA), and tyrosinemia, were analyzed separately. Although the Phe concentrations recorded at the initial dried blood spot (DBS) were observed to be elevated in individuals admitted during the neonatal period, no statistically significant disparity was identified among the initial Phe measurements collected in our center. In the cohort of LBW and preterm neonates, a notable decline in phenylalanine concentrations was documented upon their admission to our center. The preliminary phenylalanine concentrations were markedly elevated in neonates with a prior history of jaundice; however, there was no discernible difference between the groups when assessed regarding the administration of phototherapy.



Diagonal segments are produced by ties.

Supplementary Fig. S1. Receiver operating characteristic (ROC) curve analysis for predicting early admission to the metabolic clinic, based on the 1st dried blood spot phenylalanine level yielded a cut-off value of 4.05 mg/dL (area under the curve: 0.816, 95% confidence interval: 0.777–0.854, $p < 0.001$, sensitivity: 90.2%, specificity: 68%).