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Impact of Longitudinal Plasma Leucine Levels on the Intellectual Outcome in Patients with Classic MSUD

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ABSTRACT: Maple syrup urine disease (MSUD) is an inherited deficiency of branched chain α-ketoacid dehydrogenase (BCKDH) activity impairing the degradation of the branched chain amino acids valine, leucine, and isoleucine. Classic MSUD may lead to severe neonatal encephalopathy including coma and impaired cognitive outcome in later life. Early start of dietary treatment and careful metabolic control may improve the outcome of patients with classic MSUD. The aim of this study was to investigate the impact of long-term metabolic control assessed by plasma leucine levels on cognitive outcome in patients with classic MSUD. Plasma leucine levels of 24 patients were obtained retrospectively for the first 6 y of life and yearly medians of mean plasma leucine levels were calculated. At the age of 6 y, IQ tests were performed. Yearly medians of mean plasma leucine levels yielded three homogeneous clusters (low, intermediate, high). Patients of the low cluster showed statistically significant higher IQ scores compared with those of those of intermediate and high clusters. Long-term plasma leucine levels are associated with impaired cognitive outcome in patients with classic MSUD. To achieve the best possible intellectual outcome for affected individuals, we recommend that in infants and preschool children the target range for plasma leucine should not exceed 200 μmol/L. (***Pediatr Res* 59: 17–20, 2006**)

MSUD (OMIM 248600) is an autosomal recessive inherited disorder affecting the metabolism of the branched-chain amino acids (BCAAs) valine, leucine, and isoleucine. The estimated incidence of this panethnic disorder is around 1:250 000 in Germany (1) but may be much higher in populations with a high rate of consanguineous marriages (2) or low gene shifting (3). In MSUD, the BCKDH complex is impaired and high concentrations of branched-chain amino and the corresponding 2-keto acids accumulate in patients on an unrestricted diet or during catabolic episodes. Leucine and its transamination product 2-keto isocaproic acid are thought to have potential neurotoxic effects leading to acute and chronic brain dysfunction (4), and evidence of cellular toxicity has been obtained from a neuronal cell model with decreased BCKDH activity (5).

Clinicians distinguish between the most severe classic form of MSUD with onset of encephalopathy in the first week of life and milder variant forms with later onset or even absence of cerebral symptoms. Newborns with classic MSUD are asymptomatic at birth but may develop lethargy and poor feeding within days. Untreated patients fall into coma within the first 10 d of life.

Newborn screening for MSUD by tandem MS technology has been started in various countries in 2000. Until then, infants with classic MSUD had most frequently reached a critical comatose state when the disorder was suspected. Diagnosis usually was made beyond 10 d of life due to highly increased plasma concentrations of the BCAAs. Patients then required rapid removal of the toxic substances by invasive measures, *e.g.* hemofiltration (6,7). Once the infants have passed this critical condition they need a permanent dietary reduction of BCAA intake to prevent re-intoxication. Adequacy of dietary BCAA intake is assessed by regular measurement of plasma BCAA levels.

Without early and effective treatment children develop severe and permanent brain damage including spasticity or they even die within the first months of life (3). It has been shown that severe neonatal manifestation of classic MSUD leads to the development of impaired cognitive function in later life. Furthermore, intellectual outcome has been found to be inversely correlated with the duration of elevated plasma leucine levels during the neonatal period (8–10). In the few MSUD patients who received an immediate postnatal treatment (subsequent children in families at risk), some developed cognitive deficits in later life despite the prevention of any neonatal illness (8). Thus, additional factors besides plasma leucine levels during the neonatal period may contribute to the development of intellectual performance in later life.

This paper aims to describe a possible influence of longitudinal plasma leucine levels in childhood on the intellectual outcome in patients with classic MSUD.

METHODS

Patients. Twenty-four patients with classic MSUD (10 females, 14 males) followed by different pediatric metabolic centers in Germany, Austria, and Switzerland were enrolled in the study. All patients had reached or already passed the age of 6 y and all were treated with a BCAA-restricted diet.

Twenty-one patients had presented with neonatal encephalopathy and had plasma leucine levels higher than 1000 μmol/L for a mean duration of 15.5 ± 3.1 d (range 11–21 d). Peak plasma leucine levels ranged from 1240 to

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Abbreviations: BCAA, branched chain amino acid; **BCKDH,** branched-chain 2-keto acid dehydrogenase; **MSUD,** maple syrup urine disease; **WISC-R,** Wechsler Intelligence Scale for Children–Revised

4505 μmol/L (2739 ± 790). After the resolution of neonatal illness, none had neurologic symptoms.

Three younger siblings of affected patients with MSUD have been treated prospectively after birth. These patients had no neonatal encephalopathy at all. In one patient, plasma leucine concentration never exceeded 1000 μmol/L. In two patients, plasma leucine did not exceed 1150 μmol/L and decreased to 1000 μmol/L by d 4 and d 8, respectively.

Details of the medical history and the long-term metabolic control were taken from the patients' medical files. In particular, for every patient, the postnatal time during which plasma leucine levels remained above 1000 μmol/L was noted. All available plasma concentrations of leucine, valine, and isoleucine were taken from the original laboratory reports. Only plasma leucine concentrations measured by column chromatography were accepted. If several determinations of amino acids were performed within 1 d, only the highest value was used.

At a mean age of 6 y, IQ scores were either obtained by the nonverbal Snijders-Oomen-Test, the Kaufmann Assessment Battery for Children, or the revised Wechsler Intelligence Scale for children. Part of the IQ scores were previously published by Hilliges *et al.* (8). Data obtained by the Kramer test (three additional patients) were not used for statistic analysis due to a lack of comparability with the other tests. In four patients, no evaluation of cognitive capacity had been performed. However, data for these patients were used to compare long-term plasma leucine levels and for cluster analysis.

All patients gave informed consent, and the study was approved by the local ethic review board.

Calculations and statistics. More than 12,000 plasma leucine values were appraised with a mean of 32 ± 14 analyses per patient and year. For each patient, the yearly median values of all quantitative plasma leucine levels were calculated. The courses of the first 6 yearly medians were grouped by "hierarchic cluster analysis" using Ward's procedure. The squared Euclid distance was used as distance dimension. Results of the cluster analysis were validated using the discriminant analysis. The IQs of the different clusters were compared using the Kruskal-Wallis test. Differences showing a p value <0.05 were regarded as statistically significant. All statistical analysis were performed using the SPSS program.

RESULTS

The patients' mean age at the time of evaluation was 13.1 (± 6.5) y. The yearly medians of plasma leucine levels are compiled in Table 1. The profiles of the first six yearly medians were grouped by cluster analyses into three clusters, designated as low, intermediate, and high (Fig. 1).

Patients in the low cluster ($n = 8$) showed a mean of yearly median plasma leucine level of 189 ± 82 μmol/L, which is approximately 1.2-fold of the upper normal value (Table 2; range of normal values is 77–153 μmol/L) (11).

The intermediate cluster included 13 patients with a mean of yearly medians of plasma leucine concentration of 379 ± 147 μmol/L and thus not exceeding threefold of normal values (Table 2).

The high cluster consisted of only three patients. The mean of median plasma leucine levels was 572 ± 217 μmol/L, which is approximately 4.5-fold of normal values (Table 2).

Interestingly, the means of the median plasma leucine levels in the high and intermediate clusters were nearly identical during the first 2 y of life. From y 3 onward, the means of the yearly median plasma leucine levels differed between the two clusters (Fig. 2). No significant differences were found for either age at the time of evaluation or for gender between the patients of the three clusters.

Cognitive outcome and relationship with metabolic control. In total, the IQ scores of 15 patients were obtained for statistic analysis (five in the low cluster, eight in the intermediate cluster, and two in the high cluster (Table 3). The patient's IQ scores were related to longitudinal plasma leucine concentrations. For the three clusters, the mean IQ scores showed an inverse relationship to the average plasma leucine levels and thus to the quality of metabolic control.

Median IQ score of patients in the low cluster was 102 (range, 86–108). In the intermediate cluster, it was 76 (range, 57–99), and in the high cluster, it was 57 (range, 50–64). The differences of the IQ scores between the three clusters were statistically significant ($p < 0.05$; Fig. 2).

Table 1. *Yearly medians of plasma leucine levels for 24 patients with MSUD over 6 y*

	Yearly medians of plasma leucine concentration (μmol/L)					
Patient	1st y	2nd y	3rd y	4th y	5th y	6th y
1	302	321	420	187	336	443
2	290	328	298	298	382	412
3	187	208	183	282	221	210
4	130	252	412	466	321	351
5	375	527	462	553	908	695
6	275	481	573	626	733	546
7	321	271	443	336	450	538
8	191	321	397	374	450	450
9	363	527	573	519	611	191
10	183	198	332	384	377	426
11	282	557	294	496	500	515
12	305	313	183	218	176	237
13	271	378	344	656	374	485
14	208	593	58	98	379	755
15	168	168	397	336	580	476
16	88	84	179	134	221	221
17	466	901	134	439	267	322
18	92	115	176	103	92	172
19	313	252	263	282	114	142
20	256	105	116	322	266	195
21	340	202	343	80	98	250
22	563	298	359	466	412	401
23	115	74	68	79	99	291
24	244	153	763	649	862	863

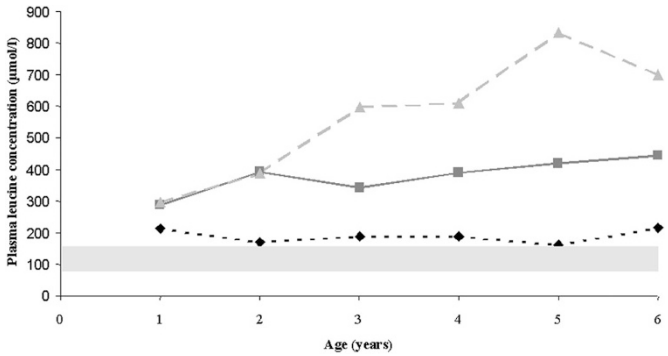

Figure 1. Plasma leucine levels in 25 patients with classic MSUD over 6 y. Means of yearly median plasma leucine levels (μmol/L) for 24 patients with MSUD according to the three clusters. The low cluster (◆) comprises eight patients, the intermediate cluster (■) 13 patients, and the high cluster (▲) three patients, respectively. Shading shows normal range of plasma leucine concentration.

DISCUSSION

This study provides evidence that the long-term metabolic control of patients with the classic form of MSUD has a major impact on the patient's cognitive outcome.

Table 2. *Means (± SD) of yearly median plasma leucine levels over 6 y according to the three clusters*

	Means (± SD) of yearly median plasma leucine levels (μmol/L)					
Cluster	1st y	2nd y	3rd y	4th y	5th y	6th y
Low	212 (±105)	169 (±87)	189 (±84)	188 (±100)	161 (±69)	215 (±46)
Intermediate	288 (±123)	393 (±201)	343 (±132)	389.0 (±145)	418 (±99)	443.0 (±130)
High	298 (±68)	387 (±204)	599 (±152)	609 (±50)	834 (±91)	701 (±159)

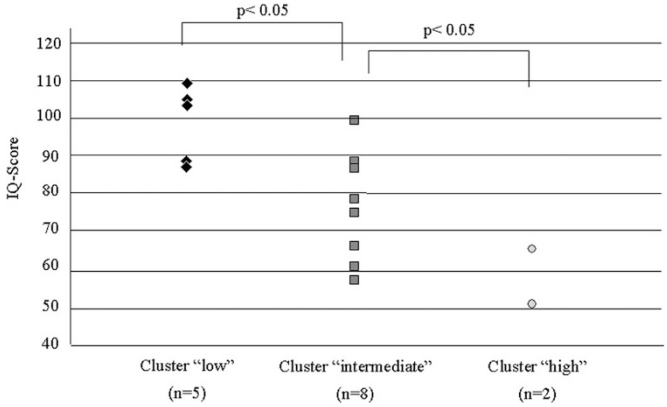

Figure 2. IQ scores of patients with MSUD in the three clusters ($n = 15$). Patients with best metabolic long-term control showed best cognitive capacity. Patients with poorer metabolic long-term control scored significantly lower on IQ.

Table 3. *Plasma leucine levels and IQ performance in the three clusters of patients with MSUD*

Cluster	Peak plasma leucine concentration (μmol/L)	Duration of plasma leucine > 1000 μmol/L (d)	IQ	Age at IQ testing (y)
I	3411	13	*	*
	4505	17	102	4
	[1145]	[4]	†	†
	3450	14	108	7
	2824	13	104	7
	2336	15	†	†
	2733	14	87	7
	1700	14	86	7
II	[1122]	[8]	[57]	[13]
	2573	21	60	13
	2480	12	99	8
	3618	19	57	6
	1496	16	77	6
	3794	11	65	6
	1481	11	*	*
	3344	16	87	4
	2878	11	†	†
	1240	21	*	*
	2644	18	86	10
	2560	17	75	6
	2744	20	**	**
III	2745	16	64	8
	[802]	[1]	[65]	[7]
	2870	15	<50	6

* Only results from Kramer test were documented when no comparison with the other IQ test was possible.

† No IQ test performed.

Numbers in brackets: prospectively treated patients, IQ scores not used for statistic analysis.

Irrespective of neonatal encephalopathy, normal cognitive outcome is achievable if long-term plasma leucine levels are close to normal. However, in patients without neonatal encephalopathy and thus with optimal starting-point conditions, intellectual outcome seems to be heavily influenced by long-term plasma leucine levels.

The cohort of patients with MSUD described here is relatively large compared with the incidence of the disease and the number of patients attending each single metabolic centre. Nevertheless, the potential of statistical examinations is limited by the overall small number of 24 patients. Despite these limitations we tried to identify influencing and/or predicting factors for the outcome of MSUD.

All patients were born, diagnosed, and treated in Austria, Switzerland, or Germany. However, their parents were immigrants in 62.5%, whereas in 37.5%, the parents were from the native population. Children from immigrant families are clearly underrepresented in the low cluster (37.5%) and are more frequent in the intermediate and high clusters. Possible explanations for that may be distinct cultural and health beliefs as well as linguistic deficiencies in the migrant community. Therefore, proper communication on the basics, characteristics, and treatment measures of that disease between the therapeutic team and the patient's family is essential. The use of written instructions in the patient's and his or her parents' primary language may be helpful in maintaining better outcome. Staff with adequate language skills and knowledge of cultural understanding is also preferred.

Despite the fact that all patients received treatment in German-speaking countries, there may be differences in the treatment recommendation among the individual metabolic centers regarding the long-term plasma leucine levels that should be appreciated. Hence, patients could have been assigned *a priori* to a specific cluster.

Interestingly, the high cluster comprised more patients who were older (not significant). Yet, it remains open whether the younger patients had more benefit from improved knowledge of the treatment of MSUD. Another explanation could be that with age and independence from parental surveillance, dietary control became worse.

Finally, children with moderate and poor long-term metabolic control had more severe metabolic decompensations than patients with excellent metabolic long-term control (data not shown). This may suggest that knowledgeable parents may recognize an imminent derangement during catabolic stress in time and start preventive dietary measures early.

Previous authors have indicated that early and meticulous treatment of patients with MSUD can result in normal intellectual outcome (9). However, Kaplan and colleagues (9) unfortunately mixed developmental quotients obtained from

the Mental Scale of the Bayley Scale of Infant Development with "real" IQ test results from Stanford-Binet or WISC-R.

Additionally, there is a clear correlation between the duration of plasma leucine levels above 1000 μmol/L in the neonatal period and intellectual outcome (8,10). Nevertheless, early diagnosis of MSUD and rapid lowering of plasma leucine concentrations to less than 1000 μmol/L in neonates are not the only crucial points for good intellectual performance in later life. The long-term biochemical control is another, and maybe the most important, factor that has an extremely important influence on the intellectual outcome in children with MSUD. Kasinski *et al.* (5) have identified leucine as the major cell toxin in neuronal cells with impaired BCKD activity. Araujo and coworkers (12) speculated that a decrease of essential amino acids in brain may lead to reduction of protein and neurotransmitter synthesis in MSUD. However, these observations derive from an animal model and the authors point out that the relevance for MSUD patients yet has to be evaluated. Additionally, Zielke *et al.* (13) investigated the competition for the transport of large neutral amino acids across the blood-brain barrier as a possible pathomechanism in MSUD. Recently, Yudkoff *et al.* (14) reported on a depletion of different amino acids in the brain, including glutamate, glutamine, aspartate, and alanine. The authors concluded that this depletion results in a compromise of energy metabolism and a diminished rate of protein synthesis. In summary, it becomes clear from all these reports that long-term metabolic control is essential for normal brain development and best possible neurocognitive outcome.

To enable parents and patients to come up with the best treatment results, clear and comprehensive information on treatment, including the target range for plasma leucine levels, and continuous training is essential. Furthermore, regular biochemical monitoring and early and sufficient intervention during catabolic episodes is mandatory.

The present retrospective study shows that in infants and preschool children the target range for plasma leucine should not exceed 200 μmol/L to achieve the best possible intellectual performance. This may not be achieved during catabolic episodes that may occur during intercurrent illnesses but should be the goal for long-term treatment.

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