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Branched-chain α-ketoacid dehydrogenase deficiency (maple syrup urine disease): Treatment, biomarkers, and outcomes

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ABSTRACT

Over the past three decades, we studied 184 individuals with 174 different molecular variants of branched-chain α-ketoacid dehydrogenase activity, and here delineate essential clinical and biochemical aspects of the maple syrup urine disease (MSUD) phenotype. We collected data about treatment, survival, hospitalization, metabolic control, and liver transplantation from patients with classic (i.e., severe; n = 176), intermediate (n = 6) and intermittent (n = 2) forms of MSUD. A total of 13,589 amino acid profiles were used to analyze leucine tolerance, amino acid homeostasis, estimated cerebral amino acid uptake, quantitative responses to anabolic therapy, and metabolic control after liver transplantation. Standard instruments were used to measure neuropsychiatric outcomes. Despite advances in clinical care, classic MSUD remains a morbid and potentially fatal disorder. Stringent dietary therapy maintains metabolic variables within acceptable limits but is challenging to implement, fails to restore appropriate concentration relationships among circulating amino acids, and does not fully prevent cognitive and psychiatric disabilities. Liver transplantation eliminates the need for a prescription diet and safeguards patients from life-threatening metabolic crises, but is associated with predictable morbidities and does not reverse pre-existing neurological sequelae. There is a critical unmet need for safe and effective disease-modifying therapies for MSUD which can be implemented early in life. The biochemistry and physiology of MSUD and its response to liver transplantation afford key insights into the design of new therapies based on gene replacement or editing.

1. Introduction

In 1954, Menkes and colleagues described four siblings who were born healthy but developed encephalopathy within the first week of life and died by age three months with cerebral edema and urine odor "strikingly similar to that of maple syrup" [1]. Branched-chain α-ketoacid dehydrogenase (BCKD) deficiency, more commonly known as maple syrup urine disease (MSUD; MIM 248600), was subsequently traced to biallelic mutations in one of three genes (*BCKDHA*, *BCKDHB*, *DBT*) which encode subunits of the multimeric mitochondrial complex

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that decarboxylates α-ketoacid derivatives (BCKAs) of the branched-chain amino acids (BCAAs): leucine, isoleucine, and valine.

MSUD affects ~1 per 150,000 newborns in outbred populations [2,3] but is enriched within certain endogamous groups [4,5]. Among North American Old Order Mennonites, severe ('classic') MSUD affects as many as 1 per 400 births due to a founder variant of *BCKDHA* (c.1312T > A, p.Tyr438Asn) which has drifted to a high carrier frequency (~10%) in certain extant demes. The Clinic for Special Children (CSC) is sited in rural Pennsylvania, a region densely populated with Mennonites, and since 1989 has drawn an ethnically and genetically diverse group of MSUD patients from 25 US states and seven countries.

Classic MSUD is among the most volatile and dangerous inherited metabolic conditions: acute elevations of leucine and α-ketoisocaproic acid (αKIC) cause metabolic encephalopathy and critical brain edema, whereas chronic amino acid and neurotransmitter imbalances pose risk for intellectual disability, executive dysfunction, and psychiatric illness (Fig. 1) [6]. Dietary therapy is challenging to implement and management of each metabolic crisis is precarious and complex [7]. Orthotopic liver transplantation restores BCAA homeostasis but introduces short- and long-term health risks [8]. Thus, there remains a pressing need for better, safer, disease-modifying therapies.

Gene replacement and editing technologies hold promise [9], but a paucity of natural history and biomarker data can impede the design of clinical trials for rare disorders [10,11]. The CSC cohort closes this gap, representing a broad spectrum of MSUD patients followed prospectively at a single center for three decades. Here, we draw on this experience to

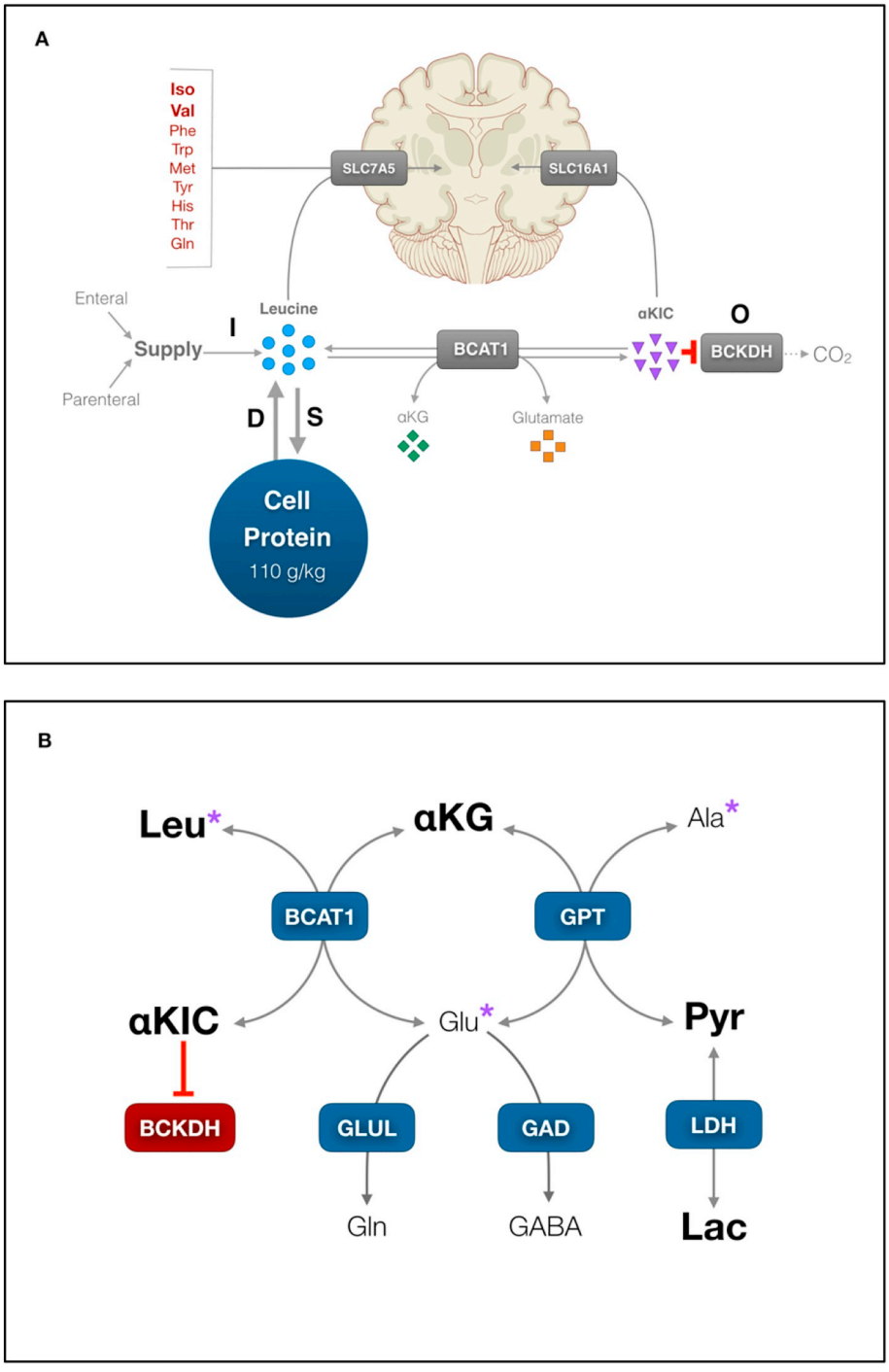


Fig. 1. Pathophysiology of maple syrup urine disease (A) In patients with MSUD, branched-chain α-ketoacids cannot be oxidized (O) by the dehydrogenase complex (BCKDH) and leucine tolerance (I) reflects unmeasured ('insensible') protein losses and the balance between endogenous protein synthesis (S) and degradation (D). Leucine comprises ~10% of tissue protein (~110 g/kg of body weight) and can increase rapidly during catabolic states, altering brain chemistry by competing with nine other amino acids for entry into the brain via the facilitative SLC7A5 transporter. Branched-chain amino acid transaminase (BCAT1) catalyzes the formation of α-ketoisocaproic acid (αKIC) from leucine and α-ketoglutarate (αKG); αKIC enters brain via the monocarboxylate transporter (SLC16A1) and is neurotoxic at high concentrations. (B) Elevated tissue αKIC reverses normal flow through BCAT1, depletes tissues of glutamate (a substrate for glutamine and γ-aminobutyric acid [GABA]), and indirectly drives flux through glutamate-pyruvate transaminase (GPT; a.k.a. ALT, SGPT) to form pyruvate from α-ketoglutarate (αKG) and alanine (Ala). In patients with classic MSUD, these interconversions explain inverse relationships of leucine to glutamate, glutamine, and alanine, and likely underlie the depletion of glutamate and elevation of lactate observed in brain tissue during metabolic encephalopathy (purple asterisk traces the normal physiologic flow of leucine-derived nitrogen). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

describe key elements of the MSUD phenotype and delineate essential outcome measures for clinical trials.

2. Methods

2.1. Patients and clinical methods

Using CSC clinical databases and Mennonite historical records [12], we identified 190 individuals with a clinical diagnosis of MSUD. Reliable data were available for 184 of them, 175 of whom had molecular confirmation of MSUD by sequencing of *BCKDHA* (n = 135), *BCKDHB* (n = 26), or *DBT* (n = 14) in the CSC core laboratory (Table S1). The study was conducted under Penn-Lancaster General Hospital IRB protocol 2008–095-CSC. All surviving patients or their parents consented in writing to participate.

We analyzed a total of 13,589 amino acid profiles collected in clinical (plasma) or home (dried filter paper blood spots) settings and comprised of samples from patients with biallelic *BCKDHA* c.1312 T > A mutations (n = 12,042), other classic genotype combinations (n = 1024), intermediate MSUD (n = 159), or following liver transplantation (n = 364). Sample timing was intentionally random to capture the full variation of plasma amino acid values in relation to feeding, fasting, and illness. Blood amino acid concentrations were compared to those from a control population of 52 Amish and Mennonite children not affected by disorders of amino acid or organic acid metabolism and reported, unless otherwise noted, as the mean value ± one standard deviation (SD).

Half of the amino acid profiles (n = 6784) were collected from a subgroup of 41 *BCKDHA* c.1312 T > A homozygotes (~165 samples per patient) born between 2005 and 2018 who were treated prospectively with a single line of medical foods (Nutricia North America) enriched with seven amino acids (see below) that compete with BCAAs for cerebral uptake across the blood-brain barrier via the SLC7A5 (a.k.a. LAT1) transporter. The formulation intended for infants (Complex Junior MSD) was found to be safe and well-tolerated in a clinical trial [13]. Between 12 and 24 months of age, patients were transitioned to a second formulation (Complex Essential MSD) with a different vitamin blend and twice the amino acid content. An MSD Amino Acid Blend was prescribed on a limited basis to some older patients seeking weight control.

Episodic elevations of leucine were managed at home using a 'sick-day' diet recipe devoid of leucine and high in calories, BCAA-free amino acids, isoleucine, and valine [7]. Patients hospitalized for metabolic encephalopathy were treated according to a standard inpatient protocol (Table 1) using a custom MSUD total parenteral nutrition (TPN) solution that could be formulated on-demand at Penn Medicine-Lancaster General Hospital inpatient pharmacy. Continuous intravenous insulin was used to maintain euglycemia and optimize protein anabolic rates during hyperalimentation. Intravenous infusions of isoleucine and valine (20–120 mg/kg·day each) were used in parallel with TPN to optimize the anabolic rate of leucine utilization. In severely intoxicated patients, mannitol (10%), hypertonic saline (3%), and furosemide were administered judiciously to prevent extracellular hypo-osmolality and mange brain edema [7]. Enteral (or parenteral) leucine was typically reintroduced when the plasma concentration decreased to < 100 μmol/L.

2.2. Metabolic phenotyping

2.2.1. Dietary leucine tolerance and protein turnover

Leucine tolerance is defined as the weight-adjusted daily leucine intake that is sufficient for normal growth and maintains plasma leucine concentration within the normal reference range (mean ± 2SDs). In persons with classic MSUD, in vivo oxidation and urinary losses of BCAAs are negligible [14,15]. Thus, leucine tolerance reflects a balance between unmeasured protein losses (e.g., sloughed skin, hair, and nails) and the net accretion of body protein, which in turn is linked to growth rate [16]. During metabolic crises, changes of plasma leucine trace whole body protein turnover, which can be quantified if one assumes the human body is 10–12% protein [17], protein is 7–8% leucine by weight [18,19], and free leucine (molecular weight 131 mg/mmol) is evenly distributed in total body water (Fig. 1) [20]. In other words,

Table 1
Inpatient management of MSUD metabolic crisis.

Primary Clinical Goals
- Decrease plasma leucine concentration by 500–1000 μmol/L per 24 h.
- Maintain serum osmolality within the normal range (~285–300 mOsm/kg H_2O) and prevent it from decreasing > 0.20 mOsm/kg H_2O per hour (> 5 mOsm/kg H_2O per day)
- Maintain serum sodium concentration of 138–145 mEq/L with minimal fluctuation
- Monitor for and treat signs of intracranial hypertension and impending brain herniation
- Anticipate and prevent iatrogenic abnormalities associated with high infusions of fluid, glucose, and insulin (e.g. hyperglycemia, hypoglyemia, hyponatremia, hypokalemia, hypophosphatemia)

General Measures
- Identify and treat precipitating catabolic stressors (e.g., infection, dehydration, trauma)
- Establish euvolemia using *isotonic* sodium chloride solutions
- Establish central venous access
- Schedule antipyretics (e.g. acetaminophen, ibuprofen, ketorolac[a]) to control fever
- Administer antiemetics (e.g., ondansetron) to control nausea and vomiting
- *Limit* use of glucocorticoids and vasoactive catecholaminergic agents
- *Alleviate* agitation and physical pain

Metabolic Treatment
- Provide 1.5- to 3-times estimated energy expenditure (EER) as dextrose (50–70%) and lipid (30–50%)[b]
- When central access allows, use 25% dextrose solutions to minimize complications of hypervolemia
- Continuous insulin infusion: 0.02–0.15 units/kg·hour; titrate to maintain blood glucose[c] 100–160 mg/dL
- Total protein equivalent intake (enteral + parenteral)[d]: 2.0–3.5 g/kg·day as BCAA-free amino acids
- Isoleucine and valine supplements (enteral + parenteral)[e]: 20–120 mg/kg·day *each*; titrate to plasma concentrations of 400–800 μmol/L

Cerebral Edema
- Monitor for signs of intracranial hypertension and impending brain herniation
- Administer hypertonic (3%) saline drip: 2–10 mEq/kg·day sodium chloride; titrate to serum osmolality 285–300 mOsm/kg H_2O, serum sodium 138–145 mEq/L, and serum osmolality *change* ≤ 0.2 mOsm/kg H_2O·hour (≤ 5 mOsm/kg H_2O·day)
- Treat symptomatic hypo-osmolality or worsening signs of intracranial hypertension using the following agents alone or in sequence: mannitol 0.5–1 mg/kg·dose; hypertonic (3%) saline 2–3 mEq/kg·dose; furosemide 0.5–1.0 mg/kg·dose
- For obtunded patients with cerebral edema, consider endotracheal intubation for airway protection and neurosurgical consultation to consider intracranial pressure monitoring, active CSF drainage, etc.

Laboratory Monitoring
- Serum (and/or point-of-care) glucose every 4–6 h
- Serum osmolality and electrolytes every 6–12 h
- Plasma amino acids, serum phosphorus and magnesium every 12–24 h
- Serum lipase, amylase, and transaminases every 24–48 h

[a] Ketorolac has potent effects on renal blood flow; it is contraindicated in patients who are dehydrated, known to have kidney disease, or taking other medications that affect renal perfusion.

[b] In older children and adults, calorie intakes 3-times EER (i.e., 6000 cal per day) are sometimes necessary to drive a net shift to protein anabolism and necessitate central venous access.

[c] Blood glucose can be measured from serum samples or using reliable point-of-care methods.

[d] Parenteral MSUD amino acid solutions are available from only a very limited number of specialty pharmacies, and often prove difficult to procure in a timely manner. For patients of any age who can tolerate enteral feeding (even if intubated), continuous nasogastric delivery of a BCAA-free MSUD formula (0.7–1.2 kcal/mL; 30–60 mL/h) supplemented with 1% liquid solutions of isoleucine and valine can be an effective way to meet protein equivalent goals while providing additional calories in the form of intravenous dextrose and/or lipid.

[e] For parenteral administration, isoleucine and valine are each prepared as separate 1% solutions in normal saline.

leucine represents ~1% of total and newly accreted body mass.

2.2.2. BCAA homeostasis

The wild type BCKD complex maintains tight stoichiometric relationships among the three BCAAs, such that plasma concentration ratios (μmol/L:μmol/L) of leucine to isoleucine (Leu/Iso) and valine to leucine (Val/Leu) remain close to 2.0 in diverse physiological contexts, including overnight fasting, protein loading, and catabolic illness. In contrast, these concentration ratios vary across several orders of magnitude in patients with classic MSUD [8].

2.2.3. Alloisoleucine

Alloisoleucine is a chemical derivative of isoleucine and represents the most sensitive and specific diagnostic marker for all forms of MSUD. Plasma alloisoleucine is < 5 μmol/L in healthy infants, children, and adults, and exceeds this value in 94% and 99.9% of samples from patients with intermediate and classic forms of MSUD, respectively [21].

2.3. Estimation of cerebral amino acid influx

A custom Excel spreadsheet (Microsoft Corporation) was designed to estimate the transport of BCAAs and seven other amino acids from blood to brain, as previously described [16]. Briefly, a group of ten zwitterionic amino acids (glutamine, histidine, isoleucine, leucine, methionine, phenylalanine, threonine, tryptophan, tyrosine, and valine), most of which are essential, compete for entry into the brain via SLC7A5 [22]. The transporter is saturated under physiological conditions, such that cerebral uptake of each SLC7A5 substrate is influenced by ambient concentrations of its competitors. Competition is expressed by an *apparent* K_m, called K_{app} (μmol/L), calculated for each amino acid according to the equation:

$$K_{app} = K_m\left[1 + \sum_{1\rightarrow n}(C_i + K_i)_n\right] \quad (1)$$

Where K_m is the classical Michaelis-Menten affinity constant for the single amino acid of interest, C_i is the plasma concentration (μmol/L) for each of n competitors, and K_i is the classical affinity constant of that competitor (μmol/L). For a given plasma amino acid profile, K_{app} values were determined for each SLC7A5 substrate using published Michaelis-Menten parameters [22]. The K_{app} value was then used to estimate the brain influx (nmol per minute per gram of brain tissue) of each amino acid in the competing group, according to the equation:

$$\text{Influx} = (V_{max})\,(C)/(K_{app} + C) \quad (2)$$

Where V_{max} and C are the maximal transport velocity (nmol/min·g) and plasma concentration (μmol/L), respectively, of each amino acid [23]. Estimated brain influx values were compared to those calculated from a control population (N = 52) and depicted as standard scores (i.e., z-scores), where z = [(patient value – control mean)/control standard deviation]. All cerebral uptake values represent calculated heuristics and should not be interpreted as *direct* measurements of amino acid flux.

2.4. Psychometric testing

Eighty-two classic MSUD patients had IQ testing using the Stanford-Binet Intelligence Scales, 5th Edition (SB-5) [24], which assesses full-scale intelligence quotient (FSIQ) as well as subdomains for verbal and non-verbal IQ, memory, visual-spatial, quantitative, and fluid reasoning, and fund of knowledge. The cohort tested was comprised of 30 *BCKDHA* c.1312 T > A homozygotes born between 1967 and 2004, 21 *BCKDHA* c.1312 T > A homozygotes born between 2005 and 2018 and treated prospectively with SLC7A5 substrate-enriched medical foods, and 31 non-Mennonites with classic MSUD. For a subgroup of 31*BCKDHA* c.1312 T > A homozygotes (DOB 1967–2004 [n = 15]; DOB 2005–2018 [n = 16]), IQ testing was performed on age-matched control siblings for comparison. Twenty patients (mean 12.0 ± 6.7, range 3.6–22.4 years) underwent neurocognitive testing before and ≥1 year after liver transplantation.

Diagnoses of depression, anxiety, and panic disorder were guided by the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) or its childhood version. Age-adjusted measures for depression and anxiety were drawn from the Beck Depression and Anxiety Inventories or subscores of the Beck Youth Inventories of Emotional and Social Impairment. Scaled scores for adult and youth versions were reconciled by calculating z-scores, as previously described [6].

2.5. Statistical methods

All statistical calculations were performed using Prism 8 (https://www.graphpad.com). For comparisons involving three or more groups, we used one-way analysis of variance (ANOVA) and the Tukey post-test to detect pairwise differences among groups (Tukey $p < .05$). Where matching was appropriate, intra- and intersubjective neurocognitive measures were compared using a paired, two-tailed t-test. Associations between variables were generally tested using simple linear regression or Spearman's test for correlation (r_s). The Pade approximant function in Prism 8 was used to fit plasma concentration relationships between leucine and other circulating non-essential amino acids (e.g. alanine, glutamine, tyrosine). For Kaplan-Meier analyses, death or permanent ischemic brain injury attributable to cerebral edema were considered equivalent endpoints. The Mantel-Cox log-rank test was used to detect differences (χ^2) between curves.

3. Results

3.1. Cohorts

The CSC clinical database included 184 MSUD patients (median age 18.2, range 0.1–52.9 years; 51% female) representing 3512 aggregate patient–years. For the purpose of this study, we divided them into five groups: (1) Mennonite *BCKDHA* c.1312 T > A homozygotes born between 1963 and 1988 (n = 30), prior to CSC's inception; (2) *BCKDHA* c.1312 T > A homozygotes born between 1989 and 2018 (n = 89); (3) non-Mennonites with a classic MSUD phenotype caused by various biallelic mutations of *BCKDHA*, *BCKDHB*, or *DBT* (n = 57; Table S1); (4) six patients with residual BCKD activity and an intermediate biochemical phenotype; and (5) two siblings with intermittent MSUD who were compound heterozygous for *DBT* c.75_76delAT and c.901C > T. We did not identify any individuals with *PPM1K* variants of MSUD [25]. A subgroup of *BCKDHA* c.1312T > A homozygotes born between 2005 and 2018 were treated on a consistent prospective protocol using SLC7A5 substrate-enriched medical foods as previously described [13], and were the source of data about contemporary dietary treatment and monitoring (Section 3.3).

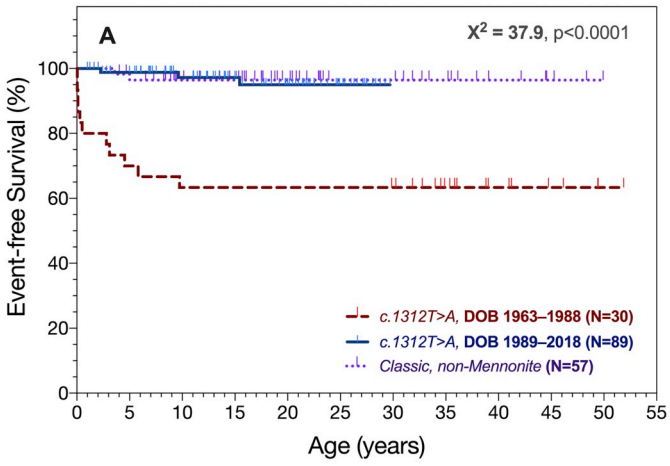
3.2. Survival, biochemical control, and metabolic crises

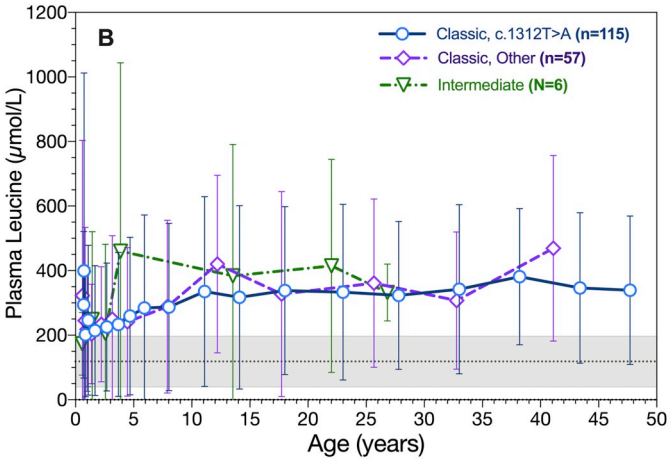
Eleven (37%) of 30 Mennonites born with classic MSUD prior to CSC's inception (i.e. birthdate 1963–1988) died from complications of metabolic encephalopathy between 36 days and 9.7 years of age (Fig. 2A). Mortality was lower ($\chi^2 = 37.9$, $p < .0001$) among 145 classic MSUD patients born after 1988: two (1.4%) died from complications of cerebral edema (ages 9.6 and 15.4 years) and one died of acute viral myocarditis (2.3 years). Event-free survival did not differ between Mennonite (n = 89) and non-Mennonite (n = 57) patients born after 1988 ($\chi^2 = 0.51$, $p = .477$).

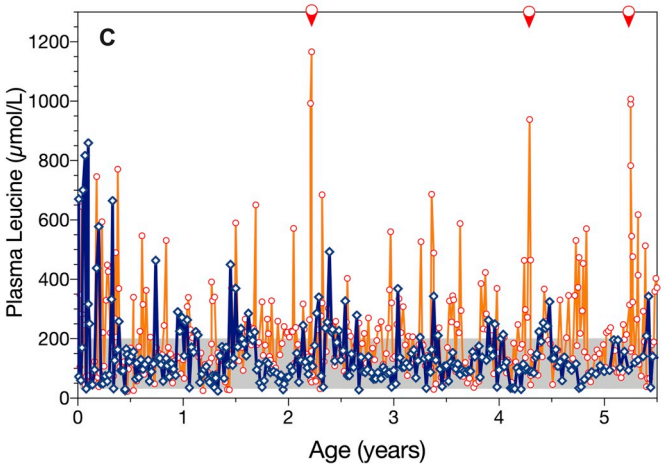
In patients with classic MSUD on dietary therapy, average plasma leucine was 282 ± 259 μmol/L (normal reference range 119 ± 38 μmol/L) (Fig. 2B) and showed considerable inter- and

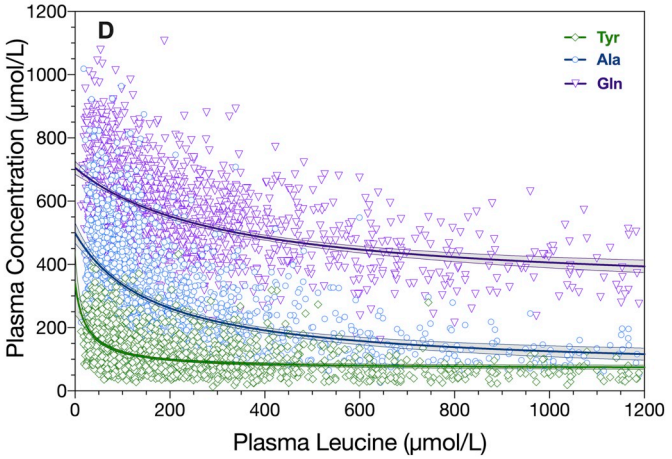
intraindividual variation (Fig. 2C). Mean BCAA concentrations did not differ between *BCKDHA* c.1312T > A homozygotes and those with other allele combinations. Average plasma leucine was similar in patients with classic as compared to intermediate MSUD (Fig. 2B, Table 2) but individuals in the latter group tolerated more intact protein, monitored plasma amino acids less frequently, and required less nutritional support during metabolic crises. Leucine showed strong inverse Spearman correlations ($p < .0001$) to multiple other circulating amino acids, most notably alanine ($r_s = -0.61$), glutamine ($r_s = -0.52$), tyrosine ($r_s = -0.49$), and threonine ($r_s = -0.47$) (Fig. 2D).

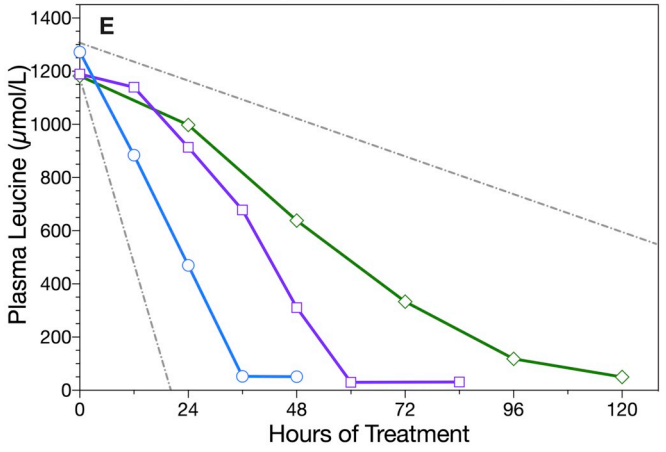
Acute metabolic intoxication necessitated 296 hospitalizations between December 1990 and May 2019. Intercurrent infection precipitated 219 (75%) of these events. Gastroenteritis (44%) was the most common admitting diagnosis but a diverse array of catabolic stresses necessitated hospitalization (Table S2). Patients were admitted at a median age of 5.6 (range birth to 39.8) years with leucine values of











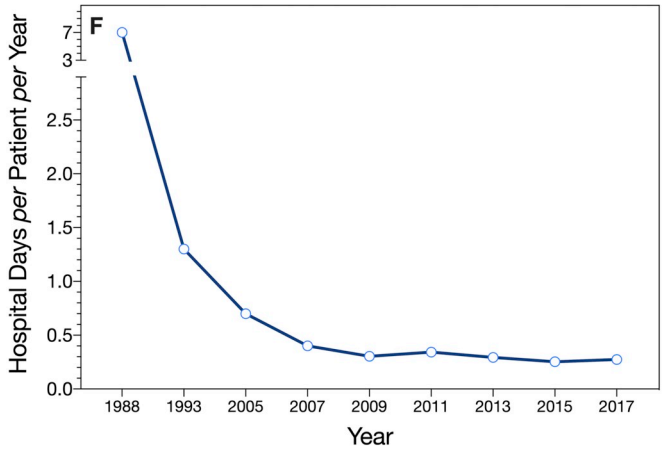


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Fig. 2. Aggregate MSUD cohort (n = 184, DOB 1963–2018): survival, biochemical control, and metabolic crises (A) Eleven (37%) of 30 Mennonites born with MSUD between 1963 and 1988 died early in life from complications of metabolic encephalopathy. Mortality was lower among 146 classic MSUD patients born after 1988 and was similar between Mennonites (n = 89) and non-Mennonites (n = 57). (B) Average plasma leucine were 1.5– to 2–times the upper limit of normal in patients with classic (blue circles, biallelic *BCKDHA* c.1312 T > A mutations; purple diamonds, other allele combinations) and intermediate (green triangles) forms of MSUD. (C) There was significant inter- and intraindividual metabolic variability, as depicted by comparing a patient with relatively tight longitudinal control (blue diamonds) to one with recurrent metabolic crises (red circles) requiring hospitalizations (red arrowheads, upper frame) (gray shaded area: mean ± 2SD normal reference range for plasma leucine). (D) Plasma leucine showed strong inverse Spearman correlations ($p < .0001$) to multiple other circulating amino acids, most notably alanine (blue circles, $r_s = -0.61$), glutamine (purple triangles, $r_s = -0.52$), and tyrosine (green diamonds, $r_s = -0.49$). (E) Between December 1990 and May 2019, there were 296 hospitalizations for metabolic intoxication, during which plasma leucine decreased 509 ± 243 μmol/kg·day to reach a median nadir of 86 (25–496) μmol/L within 2 (0.5–7) days. Different symbols-lines represent leucine curves for three hospitalized individuals and represent what clinicians can expect to observe as rapid (greed diamonds), average (purple squares), and slow (blue circles) rates of metabolic correction in response to anabolic therapy, with extreme rates for the cohort (146–1446 μmol/kg·day) indicated by gray dashed lines. The net endogenous protein synthetic rate in response to inpatient anabolic therapy averaged 0.43 ± 0.20 (0.10–1.34) g/kg·day and did not vary by age. (F) Prior to the CSC's inception, overall hospitalization rate for *BCKDHA* c.1312 T > A homozygotes was 7 hospital days/patient·year. Innovations in local monitoring and clinical care led to a steep decline after 1988 to ~0.25 hospital days/patient·year by 2005 ($p < .0001$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
MSUD biomarkers.

	Plasma Concentration (μmol/L)								Concentration Ratio (mol:mol)			
	Leucine		Isoleucine		Valine		Alloisoleucine[b]		Leucine/Isoleucine		Valine/Leucine	
	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range	Mean (SD)	Range
Control (n = 51; N = 51)[a]	119 (38)	62–200	65 (25)	26–121	208 (61)	118–335	nd		1.81 (0.31)	1.15–3.41	1.73 (0.21)	1.13–2.49
Classic MSUD (n = 12,043; N = 165)	282 (259)	2–3008	240 (190)	5–3141	495 (250)	1–3298	190 (117)	0–746	1.66 (2.24)	0.01–103.70	3.47 (4.54)	0.002–104.90
Intermediate MSUD (n = 158; N = 6)	324 (469)	12–2201	187 (250)	34–2457	428 (455)	53–5269	52 (58)	0–271	1.87 (0.84)	0.08–5.72	2.00 (1.80)	0.52–14.62
Liver Transplant (n = 241; N = 61)	187 (62)	79–422	144 (53)	40–363	324 (109)	155–639	4 (8)	0–32	1.33 (0.28)	0.90–4.00	2.05 (0.53)	1.05–5.80
One-way ANOVA P	< 0.0001		< 0.0001		< 0.0001		< 0.0001		< 0.0001[c]		< 0.0001[c]	
Tukey Pairwise Comparisons[d]												
Control vs. Classic	****		****		****		na		ns		**	
Control vs. Intermediate	****		***		****		na		ns		ns	
Control vs. Liver Transplant	ns		*		*		na		ns		ns	
Classic vs. Intermediate	ns		**		**		****		ns		****	
Classic vs. Liver Transplant	****		****		****		****		***		****	
Intermediate vs. Liver Transplant	****		ns		***		***		***		ns	

Abbreviations: ANOVA, analysis of variance; n, number of samples analyzed; N, number of patients; na, not applicable; nd, not detected; ns, not significant ($p > .05$); SD, standard deviation.

[a] 'N' indicates the number of individuals in each group; 'n' indicates the number of samples analyzed: e.g. among 165 individuals with Classic MSUD, an average of 73 blood samples per subject yielded 12,043 amino profiles for one-way ANOVA analysis.

[b] Our laboratory began quantitative alloisolecuine concentrations in recent years. Thus, sample numbers are smaller for Classic (n = 633), intermediate (n = 156), and transplanted (n = 121) MSUD patients.

[c] BCAA concentration ratios were log10 transformed for statistical comparisons.

[d] *p < .05; **p < .01; ***p < .001; ****p < .0001.

960 ± 509 (20–4812) μmol/L. Plasma leucine decreased 509 ± 243 (146–1446) μmol/L·day in the hospital to reach a median nadir of 86 (25–496) μmol/L within 2 (0.5–7) days (Fig. 2E). The average rate of endogenous net protein synthesis in response to anabolic therapy was 0.43 ± 0.20 (0.10–1.34) grams/kg·day and did not differ as a function of age ($r_s = -0.044$, $p = .523$). Total inpatient stay ranged from 0.5 to 62 days per admission. The overall hospitalization rate decreased 96% between 1989 and 2015, from 7.0 to just ~0.25 hospital days per patient per year ($p < .0001$) (Fig. 2F). We did not utilize hemodialysis to manage any metabolic crises, although this strategy can effectively reduce plasma leucine in patients with MSUD [26,27].

3.3. Contemporary dietary management

Among 41 *BCKDHA* c.1312 T > A homozygotes (DOB 2005–2018) treated prospectively from birth with SLC7A5 substrate-enriched formulas, 21 (51%) were targeted for umbilical cord molecular testing based on parental carrier status and diagnosed at an average of 12 h of life; most remained asymptomatic throughout a safe perinatal transition at home. Twenty (49%) babies were detected by state newborn screening. Neonates in this latter group were diagnosed at an average of 5 days of age and in some cases presented symptomatic with high plasma leucine concentrations (1123–2769 μmol/L); three were hospitalized between 7 and 11 days of age for metabolic encephalopathy. The remaining 17 (85%) babies diagnosed by newborn screening were managed successfully at home, provided the child had no or only mild signs of encephalopathy and could tolerate a sufficient volume of 'sick-day' formula and the parents could commit to assiduous clinical and amino acid monitoring in the outpatient setting.

Throughout infancy, energy intake from the sum of intact and BCAA-free liquid sources aligned with values predicted by the doubly-labeled water method (http://nap.edu/11537). Between 1 and 3.5 years of age, the proportion of calories from unquantified natural sources came to represent ~30% of energy intake (Fig. 3A). Weight-adjusted leucine tolerance decreased from 72 ± 13 mg/kg·day in newborns to 10 ± 2 mg/kg·day in adults (Fig. 3B) and paralleled a decrement of total weight-adjusted protein equivalent from the sum of intact and BCAA-free sources, which nevertheless remained 25–50%

higher than recommended daily allowance across the lifespan (http://nap.edu/11537). This high protein equivalent was dominated by amino acids from medical foods; the proportion of intact protein relative to BCAA-free protein equivalent decreased from ~25% during infancy to ~10% by adolescence (Fig. 3C).

Following the perinatal period, average plasma leucine (218 ± 197 μmol/L, n = 6350) was typically within two standard deviations above the reference mean until age 10 years, but 30–56% higher (313 ± 301 μmol/L, n = 434; p < .0001) and more variable (F = 2.33, p < .0001) thereafter. Parents checked an average of 64 (range 42–111) amino acid profiles during the first year of life. Sampling frequency decreased as children got older and correlated inversely with annualized average plasma leucine ($r_s = -0.41$, p = .007) (Fig. 3D). Enrichment of medical food protein with SLC7A5 substrates (phenylalanine, tryptophan, methionine, tyrosine, histidine, threonine) [13] generally maintained their plasma concentrations above the normal reference mean (Table 3) and preserved estimated average brain influx within a broad but acceptable range (Fig. 3E). However, despite 40–165% dietary enrichment relative to human milk [13], average estimated cerebral uptake of certain key amino acids

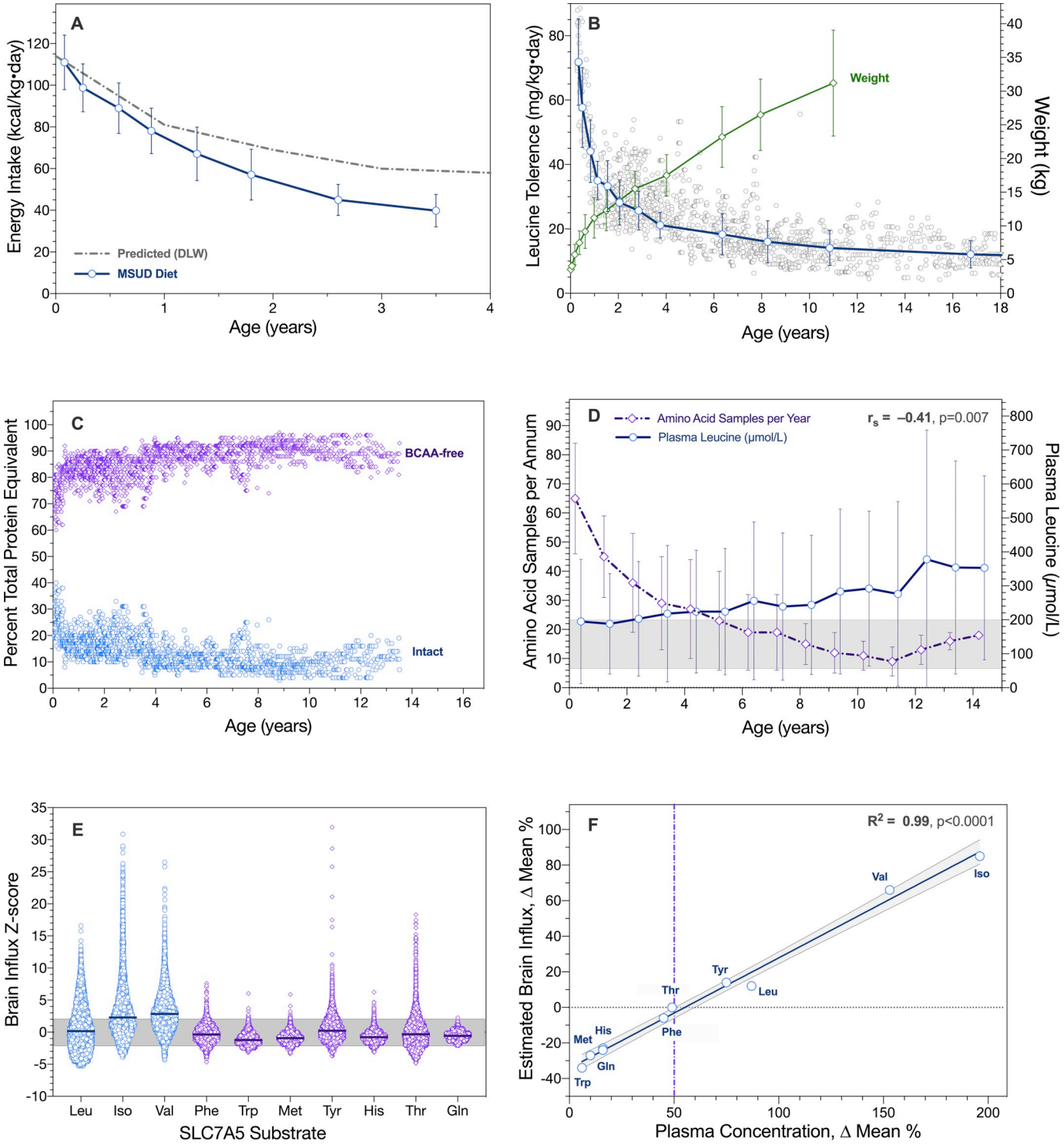

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Fig. 3. Contemporary dietary management (n = 41, DOB 2005–2018): (A) Prescribed calories from formula (blue circles) aligned with predicted values until one year of age; thereafter, calories from unquantified natural sources came to comprise ~30% of energy intake. Gray dashed line represents predicted calories based on the doubly labeled water (DLW) method (http://nap.edu/11537). (B) Average weight-adjusted leucine tolerance (blue circles) correlated with weight gain (green diamonds). (C) The proportion of ingested intact protein (blue circles) relative to BCAA-free protein equivalents (purple diamonds) decreased from ~25% during infancy to ≤15% by adolescence. The total protein equivalent from intact plus BCAA-free sources was 25–50% higher than recommended daily allowance across the lifespan. (D) Parents checked amino acid profiles most frequently during the first year of life (purple diamonds, dashed line; left ordinate axis). Sampling frequency decreased thereafter, and correlated inversely ($r_s = -0.41$, p = .007) with annualized average plasma leucine (blue circles, solid line; right ordinate axis). Values are depicted as mean ± 1SD and gray shaded area represents the normal reference range for plasma leucine ± 2SD (right ordinate axis). (E) Enrichment of medical food with seven SLC7A5 substrates (purple diamonds: phenylalanine [Phe] tryptophan [Trp], methionine [Met], tyrosine [Tyr], histidine [His], threonine [Thr], and glutamine [Gln]) maintained their plasma concentrations within 2SDs above the reference mean and partially defended estimated average brain influx. Gray shaded area represents the normal z-score range of −2 to +2. (F) For each SLC7A5 substrate, we plotted average percentage difference as compared to the normal reference mean (Δ Mean %) for its plasma concentration (abscissa) against its estimated brain influx (ordinate). In MSUD patients with chronically elevated BCAAs, the competitive transport model predicts that the plasma concentration of any competing SLC7A5 substrate must be ~50% higher than the control reference mean (purple dashed vertical line) to fully normalize its cerebral uptake (gray horizontal line). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

(tryptophan, methionine, histidine, glutamine) was nevertheless 23–34% lower than normal reference values (Table 3), reflecting strong transport inhibition by elevated BCAAs (Fig. 1A), an inverse relationship between leucine and several competing substrates (Fig. 2D), and robust oxidation of surplus dietary amino acids [28].

Prospectively treated children had normal trajectories for weight, length, and head circumference (Fig. S1). Outpatient laboratory studies showed normal serum electrolytes, creatinine, glycohemoglobin, albumin, total protein, hepatic transaminases, alkaline phosphatase, cell counts, hemoglobin, red cell indices, total iron binding capacity, ferritin, 25-hydroxyvitamin D, folate, vitamin B12, and carnitine profiles (Table S3).

3.4. Liver transplantation and biomarkers

Between 2003 and 2018, 61 (33%) MSUD patients from our cohort received an elective liver transplant at a median age of 9.7 (0.8–35.8) years under a protocol jointly developed by clinical teams at CSC and the UPMC Children's Hospital of Pittsburgh (Fig. 4A). Overall graft and patient survival were 100%. There was no survival difference between patients treated with liver transplant versus dietary therapy ($\chi^2 = 1.39$, p = .239) and the transplant rate did not differ between Mennonite and non-Mennonite individuals ($\chi^2 = 5.8$ p = .055). Thirteen (21%) liver explants from MSUD donors were successfully 'domino' transplanted into patients with other forms of liver disease [29]. Two transplanted *BCKDHA* c.1312T > A homozygotes delivered healthy babies after observing no special dietary restrictions during pregnancy.

Following liver transplantation, each plasma BCAA remained stable at 1.5- to 2-times the reference mean in the face of intercurrent catabolic stress and unrestricted natural protein ingestion (Fig. 4B, C, Table 2). Alloisoleucine proved the most discriminating biomarker of in vivo BCKD deficiency and had a linear correlation with plasma isoleucine in both classic ($R^2 = 0.50$, p < .0001) and intermediate ($R^2 = 0.67$, p < .0001) MSUD patients on dietary therapy. Alloisoleucine was ≤5 μmol/L in 77% (n = 125) of post-transplant samples (Fig. 4D). Among healthy control individuals, there was minimal variation of plasma BCAA concentration ratios (Leu/Iso 1.81 ± 0.31; Val/Leu 1.73 ± 0.21), reflecting natural stoichiometric regulation of α-ketoacid oxidation by the intact BCKD complex. These ratios varied across four orders of magnitude in patients with classic MSUD on dietary therapy. Liver transplant almost completely restored stoichiometric regulation among the BCAAs despite their persistent elevation in plasma (Fig. 4E, F).

Transplanted individuals from the present study represent 66% of the total cohort of MSUD patients (n = 93) who received an elective liver transplant at UPMC between 2003 and 2019. Among this larger group, the most common indications for early post-transplant surgical intervention were delayed wound closure (34%), chest tube drainage (15%), and ventral hernia repair (11%) (Table S4). Post-transplant exploratory laparotomies were performed for hepatic artery or graft revision (5%), hepatic artery thrombosis (5%), partial small bowel obstruction (4%), and intra-abdominal bleeding (3%). Major medical complications were acute rejection (40%), Epstein-Barr virus (5%) and cytomegalovirus (3%) disease, and immunosuppression-related lymphoproliferative disorder (3%).

3.5. Neuropsychiatric outcomes

Prospectively treated children with MSUD acquired major developmental milestones along time courses similar to their unaffected siblings (Fig. 5). Among 82 MSUD patients who underwent neurocognitive testing between 3.6 and 51.1 years of age, intelligence correlated with birthdate ($r_s = 0.39$, p = .0044) (Fig. 6A) and was on average 23% lower in a subgroup of 31 *BCKDHA* c.1312 T > A homozygotes (FSIQ 78 ± 18, range 40–113) as compared to their unaffected siblings (FSIQ 100 ± 16, range 64–144). This latter difference did not vary across subdomains and was most striking for 19 Mennonites born before 1989, who had an average FSIQ of 62 ± 17 (range 40–99), 12–60% below their age-matched siblings (Fig. 6B). Nine (42%) of these individuals also had significant static motor disability. Using incomplete retrospective data, neonatal encephalopathy was the only other variable we could clearly correlate to FSIQ (Fig. 6C). Scores on the SB-5 did not differ between Mennonite and non-Mennonite classic MSUD patients born after 1988 (Fig. 6A).

The prevalence of affective illness (depression, anxiety, and panic disorder) was alarmingly high among MSUD patients who completed appropriate testing (n = 37) and also two-fold higher among their siblings as compared to the general population (Fig. 6D). Liver transplantation did not reverse pre-existing static encephalopathy, intellectual disability, or mental illness (Fig. 6E), which appeared to drive a strong linear correlation between birthdate and age of transplant ($R^2 = 0.72$, p < .0001) (Fig. 6F).

4. Discussion

4.1. Morbidity, mortality, and longitudinal metabolic control

Before 1989, one in three Mennonite children born with MSUD died from complications of metabolic encephalopathy and the majority of survivors were permanently disabled. Early efforts at CSC to integrate metabolic services into primary care led to more rapid and affordable amino acid testing, safer outpatient management of metabolic instability, and locally accessible MSUD TPN [30]. As a result, 30-year event-free survival increased from 63% to > 95%, hospitalization rates decreased from 7 to just 0.25 hospital days per patient per year (Fig. 2A, F) [31], and key outcome determinants became clear (Table 4).

Despite these advances in clinical care, classic MSUD remains a morbid and potentially fatal disorder. The prescription diet maintains average BCAA concentrations within acceptable limits (i.e. +3SDs above the normal reference mean) but permits only ~10% of total

Table 3
Plasma concentration and estimated brain influx of SLC7A5 substrates in control subjects (N = 52) and classic MSUD patients on an SLC7A5 substrate-enriched diet (N = 41).[a]

	K_m for SLC7A5,[b] μmol/L	Medical Food Enrichment as %PE[c]	Plasma Concentration, μmol/L				Estimated Brain Influx, nmol/min·g tissue			
			Control	MSUD Diet[a]	Mean % Difference	P value[d]	Control	MSUD Diet[a]	Mean % Difference	P value[d]
			Mean (SD)	Mean (SD)			Mean (SD)	Mean (SD)		
Leucine	29	na	119 (38)	222 (205)	+87	*< 0.0001*	12.35 (2.25)	13.89 (7.95)	+12	*< 0.0001*
Isoleucine	65	na	65 (25)	193 (173)	+196	*< 0.0001*	3.53 (0.92)	6.52 (4.13)	+85	*< 0.0001*
Valine	208	na	208 (61)	523 (236)	+153	*< 0.0001*	2.50 (0.48)	4.15 (1.65)	+66	*< 0.0001*
Phenylalanine	11	+50	55 (18)	80 (35)	+45	*< 0.0001*	10.45 (1.93)	9.82 (2.57)	-6	*0.0232*
Tryptophan	50	+150	50 (18)	53 (30)	+6	*0.2396*	9.61 (2.95)	6.33 (2.73)	−34	*< 0.0001*
Methionine	31	+165	31 (10)	34 (15)	+10	*0.0366*	1.00 (0.32)	0.73 (0.25)	−27	*< 0.0001*
Tyrosine	63	+40	63 (24)	110 (65)	+75	*< 0.0001*	4.81 (1.29)	5.48 (2.59)	+14	*0.0005*
Histidine	87	+40	87 (29)	101 (40)	+16	*0.0011*	2.78 (0.89)	2.14 (0.78)	−23	*< 0.0001*
Threonine	120	+40	120 (44)	179 (95)	+49	*< 0.0001*	0.48 (0.14)	0.48 (0.25)	0	*> 0.9999*
Glutamine	527	na	527 (146)	611 (140)	+16	*< 0.0001*	1.42 (0.57)	1.08 (0.37)	−24	*< 0.0001*

Abbreviations: MSUD, maple syrup urine disease; na, not applicable; PE, protein equivalent; SD, standard deviation.

[a] Control data are from 52 healthy children without disorders of amino or organic acid metabolism and include one sample per subject. The treatment cohort consists of 41 children born between 2005 and 2018 with classic MSUD and managed consistently from birth using BCAA-free medical foods enriched with SLC7A5 amino acid substrates. For the MSUD group, a total of 6784 individual amino acid profiles (~165 per subject) were used to generate descriptive and comparative statistics.

[b] Classic Michaelis-Menten K_m values were determined empirically from studies of perfused rat brain in situ and published in Ref. [22].

[c] Enrichment as percentage of protein equivalent (PE) expresses the medical food composition of each amino acid as a percentage of total relative to the composition of human milk protein (See Ref. 13, Table 2; Strauss et al., 2010).

[d] Unpaired two-sided *t*-test with Welch's correction (not assuming equal variances). Significant ($p < .05$) differences are italicized.

nutritional support from natural sources and does not prevent life-threatening encephalopathic crises (Figs. 2B, C, and 3C). As with phenylketonuria (PKU) [32,33], the frequency of amino acid monitoring correlates with longitudinal metabolic control, both of which deteriorate with age (Fig. 3D) [34]. A minimum of 24 amino acid samples per year (i.e. one every two weeks) is necessary to optimize control of plasma leucine in older MSUD patients, none of whom actually adhere to this schedule after 9 years of age.

4.2. Major determinants of neurocognitive outcome

Even with strict dietary control and frequent amino acid monitoring, classic MSUD patients experience chronic amino acid and α-ketoacid fluctuations that predispose to cognitive and psychiatric disability via overlapping mechanisms, including: (1) abiding neurostructural effects of severe and/or prolonged neonatal encephalopathy (Fig. 6C) [12,35]; (2) chronically unbalanced amino acid transport across the blood-brain barrier (Table 3, Figs. 1A, 3E, and F) [22,36]; (3) cerebral deficiency of neurotransmitters such as glutamate, γ-aminobutyric acid, dopamine, and serotonin [6,37,38] and (4) disturbances of cerebral tricarboxylic acid flux and energy metabolism induced by αKIC (Fig. 1B) [39]. In a previous study focused on a relatively uniform subgroup of MSUD patients (N = 37) within a comparatively narrow age range (5–35 years) [6], we correlated neuropsychiatric measures with certain long-term biochemical and regional neurochemical patterns (e.g., average lifetime plasma leucine and its ratio to valine and tyrosine, as well as regional cerebral concentrations of glutamate, *N*-acetylaspartate, and creatine).

The present MSUD cohort (n = 184) is comprised of more individuals over a much wider age range (0.1–52.9 years) with remote medical records of variable quality and completeness; this limited our analysis to only coarse-grained determinants of cognitive outcome such as age and the presence or absence of neonatal encephalopathy (Fig. 6A and C). The most severe disabilities were observed among patients born before the advent of newborn screening, who commonly suffered postnatal encephalopathy lasting weeks or months (Fig. 6B) [12], had relatively infrequent amino acid monitoring [40], did not routinely receive valine or isoleucine supplements early in life [6,30], ingested formulas of variable composition quality and composition [13], and were managed without the advantages of osmotic agents or customized TPN (Table 1) [7,30]. These practice standards evolved in lockstep over a span of five decades, making it challenging to discern the effect of any one of them on outcome. Here, we can only confidently record the 'aggregate' benefit of improved clinical practices over time (Fig. 6B) [31] and observe that children born with MSUD today in resource-limited settings will face many of the same conditions and challenges that prevailed in the U.S. fifty years ago [41–43].

4.3. Dietary treatment and outcome in the modern era

In 2005, we introduced new prescription medial foods designed to counteract competitive inhibition of essential amino acid transport at the blood-brain barrier and stabilize tissue concentrations of amino acids depleted by αKIC [13]. These formulations have proven safe and well-tolerated in 41 classic MSUD patients treated continuously from birth (Fig. 3), supporting normal growth and milestone acquisition while satisfying fundamental nutritional requirements through the early developmental years (Fig. 5, Fig. S1, Table S3). Over longer periods, enriching the diet with SLC7A5 substrates increases their concentration in plasma but only partially safeguards their predicted cerebral uptake (Fig. 3E and F, Table 3). This reflects strong transport inhibition exerted by only modest elevations of leucine (K_m = 29 μmol/L) and isoleucine (K_m = 56 μmol/L) (Figs. 1A and 3F) [22], suppression of competing substrates in plasma by leucine and αKIC (Fig. 2D), and the inherent challenge of overcoming oxidation pathways evolved to maintain extracellular amino acid homeostasis in the face of broad

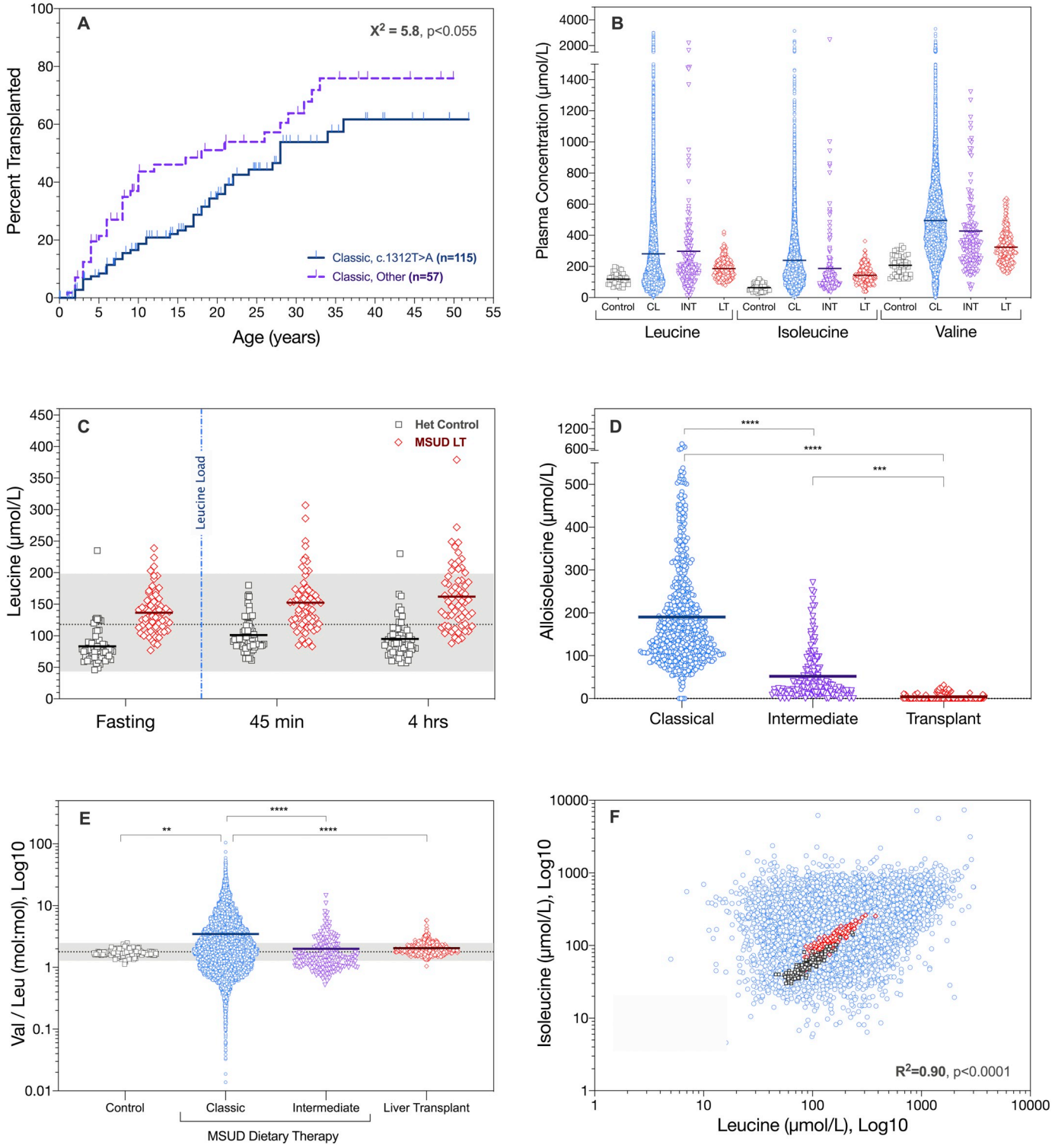

Fig. 4. Liver transplantation and biomarkers (A) Sixty-one (33%) patients received a liver transplant at a median age of 9.7 (0.8–35.8) years and transplant rate did not differ between Mennonites (solid blue line) and non-Mennonites (dashed purple line). (B) Following liver transplantation, weight-adjusted leucine intake increased 5- to10-fold as plasma BCAAs remained stable at 1.5- to 2-times the reference mean in the face of intercurrent catabolic stress and unrestricted natural protein ingestion (Control, control individuals; CL, classic MSUD on dietary therapy; INT, intermediate MSUD on dietary therapy; LT, classic MSUD patients following liver transplantation). (C) Transplanted patients with classic MSUD (red diamonds) tolerate large protein boluses, shown here by the ability to oxidize a leucine load of ~1500 mg within 45 min of ingesting a normal meal (gray squares: matched *BCKDHA* c.1312T > A heterozygous parents). In a non-transplanted patient, a similar dietary load would acutely increase plasma leucine by 400–1000 µmol/L. (D) Plasma alloisoleucine had a strong linear correlation to isoleucine in patients with classic (blue circles) and intermediate (purple triangles) MSUD on dietary therapy and was undetectable in 77% of blood samples collected after liver transplantation (red diamonds). (E) Plasma concentration ratios among the BCAAs remain within narrow limits (Leu/Iso 1.81 ± 0.31; Val/Leu 1.73 ± 0.21) among control individuals (gray squares) but vary across four orders of magnitude in patients with classic MSUD on dietary therapy (blue circles). (F) Liver transplantation (red diamonds) partially restores regulation of BCAA ratios but at concentrations approximately 2-fold higher than control values (note log10 scales, panels E and F). Asterisks represent statistically significant results of one-way ANOVA testing, with Tukey pairwise comparison p values of < 0.01(**), < 0.001 (***), or < 0.0001(****). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

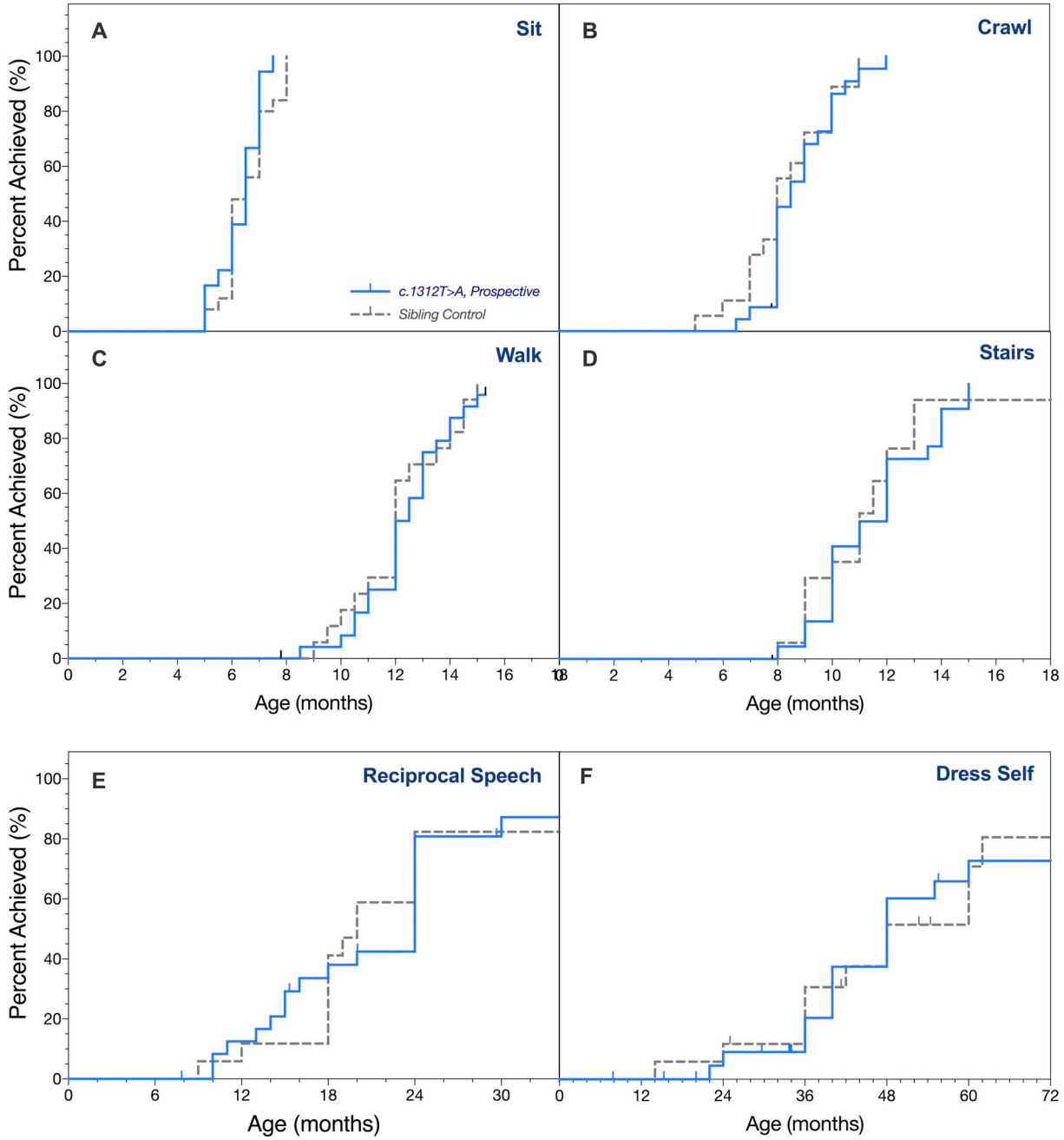

Fig. 5. Early Development. Early developmental milestone acquisition for 41 *BCKDHA* c.1312 T > A homozygotes, born 2005–2018 and treated from birth with a single line of SLC7A5 substrate-enriched medical foods. MSUD patients represented by blue solid line were compared to their siblings who did not have MSUD (gray dashed line). Milestones depicted: (A) independent sitting, (B) four-point crawl, (C) independent walking, (D) ascend stairs without assistance, (E) reciprocal verbal communication with another individual, and (F) dress self without assistance. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

dietary variation [28,44].

Unfortunately, children detected by newborn screening and treated prospectively using substrate-enriched medical foods nevertheless scored 19 ± 15% lower than their siblings on intelligence scales and were at least two-times more likely to develop mental illness (Fig. 5B and D). These observations align with findings from other published cohorts, which reveal increased risks of cognitive impairment, affective disorders, executive dysfunction, emotional strain, unemployment, and social dependence among MSUD patients managed in the modern era [34,45]. Our findings also highlight the significant psychosocial morbidity experienced by unaffected siblings (and presumably parents) of MSUD patients (Fig. 6D). We could not attribute this to heterozygosity for *BCKDHA*, *BCKDHB*, or *DBT* mutations. Rather, we believe it represents the sustained psychological stress incurred by cohabitant family members of any child afflicted with a chronic, serious, and/or life-threatening illness, as has been observed repeatedly in other contexts [46–48].

4.4. Liver transplantation and beyond

Many MSUD patients in developed countries ultimately choose orthotopic liver transplantation, which affords them an unrestricted diet and protection from metabolic crises (Figs. 4A-C). However, a significant number of transplanted patients experience surgical, infectious,

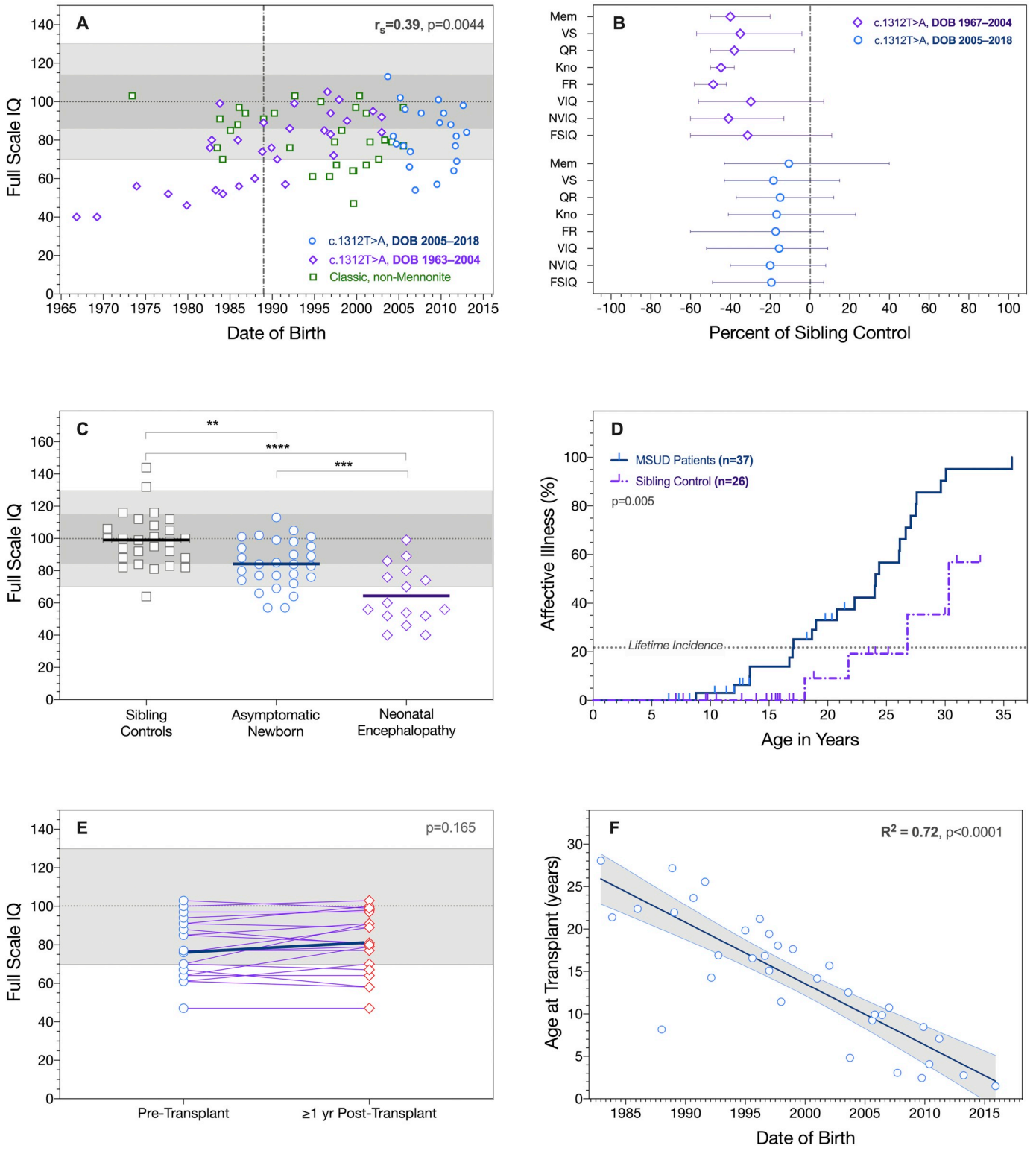

Fig. 6. Psychometric outcomes (A) Among all patients with classic MSUD, full-scale intelligence quotient (FSIQ) correlated with birthdate (r_s = 0.39, p = .0044) (purple diamonds, *BCKDHA* c.1312 T > A homozygotes born 1963–2004; blue circles, *BCKDHA* c.1312 T > A homozygotes born 2005–2018 and treated on prospective dietary protocol; green diamonds, non-Mennonite classic MSUD) and (B) was on average 23% lower in patients as compared to their unaffected siblings across subdomains (Mem, memory; VS, visual-spatial; QR, quantitative reasoning; Kno, fund of knowledge; FR, fluid reasoning, VIQ, verbal IQ; NVIQ, non-verbal IQ). (C) Neonatal encephalopathy was the only other variable we were able to correlate with FSIQ in this large cohort. (D) The probability of affective illness (depression, anxiety, and panic disorder) approached 100% by age 35 years in MSUD patients (blue solid line) and was two-fold higher among their siblings (purple dashed line) as compared to the general population (gray dotted line). (E) Liver transplantation did not reverse pre-existing static encephalopathy, intellectual disability, or mental illness, shown here as a non-significant average change (blue line, p = .165) in FSIQ among 20 MSUD patients who underwent neurocognitive testing before (blue circles, FSIQ 78 ± 15) and ≥ 1 year after (red diamonds, FSIQ 81 ± 16) liver transplant. (F) There was a strong linear correlation between birthdate and age of transplant. Asterisks represent statistically significant results of one-way ANOVA testing, with Tukey pairwise comparison p values of < 0.01 (**), < 0.001 (***), or < 0.0001 (****). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Determinants of outcome for patients with MSUD.

Neonatal Course
▪ Presymptomatic diagnosis (i.e. carrier testing, umbilical cord molecular screening)
▪ Prevention or rapid reversal of neonatal encephalopathy
Longitudinal Dietary Control
▪ Weight- and age-appropriate leucine intake based on observed tolerance
▪ Prevention of prolonged amino acid imbalances and branched-chain amino acid deficiencies
▪ Provision of sufficient essential fatty acids, vitamins, minerals, and micronutrients
▪ Frequent amino acid monitoring (via plasma or filter paper), once or twice weekly during infancy and every 1–2 weeks thereafter
Control of Metabolic Decompensation in the Outpatient Setting
▪ Convenient and affordable methods for local and/or home monitoring of metabolic status during illness
▪ Effective 'sick-day' dietary and monitoring protocols for outpatient management of intercurrent illnesses
▪ Timely access to outpatient metabolic urgent care services
Reversal of Metabolic Crises and Prevention of Neurological Sequelae
▪ Regional inpatient services with physician and nursing teams experienced in MSUD care
▪ Contemporaneous availability of MSUD total parenteral nutrition and intravenous isoleucine and valine
▪ Access to on-demand amino acid monitoring during critical illness
▪ Ability to secure central venous access for supraphysiologic calorie and dextrose administration
▪ Continuous insulin infusion and appropriate blood glucose monitoring
▪ Stringent monitoring and control of serum osmolarity and appropriate use of hyperosmolar agents and diuretics to prevent exacerbations of cerebral edema
Allogeneic Liver Transplantation
▪ Access to liver transplant centers with MSUD management experience
▪ Perioperative surgical complications
▪ Immunosuppression-related infections and/or malignancies
▪ Psychomotor disabilities and/or mental illness preceding liver transplantation

or malignant complications (Table S4) and their pre-existing neuropsychiatric morbidities do not improve (Fig. 6E) [49]. The severity of neurological disease preceding transplant is related to both the perinatal course and long-term metabolic control (Figs. 6A-C, Table 3) but neurochemical disturbances persist after liver transplantation [6], indicating that deficiency of BCKD in brain cells, which normally express 15–20% of total body enzyme activity [50], might impact cerebral metabolism and function in ways independent of circulating BCAA and BCKA concentrations.

Liver transplantation restores 9–13% of whole body BCKD activity and represents a form of gene replacement therapy for MSUD. This sets the stage for emerging DNA- and RNA-based treatment platforms. Intact allogeneic liver tissue introduces just a fraction of potential BCKD activity, most of which normally resides in human skeletal muscle (54–66%) and brain (9–20%) [50]. Thus, one can expect an incremental benefit of systemic vehicles such as adeno-associated viruses capable of targeting transgene expression to the liver, muscle, and central nervous system [9,51,52]. Newborn screening for MSUD, ubiquitous throughout the United States and many developed nations, ensures that any new disease-modifying therapy will exert its maximal clinical impact. One-time molecular therapies might be especially valuable in resource-limited settings, where therapeutic tools for managing MSUD are woefully limited and outcomes remain poor [41–43].

Disclosures

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ymgme.2020.01.006.

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