

The Glutaric Acidurias of the Amish

A Sense of Progress 1988-2011

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His mother told me - *We hoped he was not like our others. He seemed all right. He sat-up and walked when he should have. But then it happened. He was 18 months old. He had a cold and a fever. He seemed tired, so, I put him down for a nap after lunch. When I checked on him two hours later he was helpless. He couldn't hold-up his head or swallow, he couldn't sit, no strength was left. We knew then he was like the others, There was no other reason for it.*

Amos and I just sat together and cried for a long time before we took him to the doctor. We knew the doctor would send us to the hospital. We knew that after all the pain and tests and money there wouldn't be any answers and nothing could be done for him either. (The mother of an Amish infant with Glutaric aciduria in 1988, From Amos' Child)

INTRODUCTION

I am a Pediatrician. My wife Caroline and I founded the Clinic for Special Children (www.clinicforspecialchildren.org) in 1989 in Lancaster County, Pa., to provide general medical care for children with glutaric aciduria, maple syrup urine disease, and other inherited disorders of biochemistry. The Clinic was located on an Amish farm near the town of Strasburg, named after Strasbourg France.

Our non-profit medical center remains a primary care pediatric practice but it is a practice informed by genomics and it has come to be widely known as a place where emerging data about the human genome is routinely used to diagnose and care for the individual patient. Over a period of 21-years the staff of this small clinic has discovered the genetic basis of many medical problems within the Amish and Mennonite populations that present as mental retardation, seizures, dystonia, deafness, bleeding disorders, overwhelming infection, and unexpected deaths of infants and children.

These problems are not unique to the Amish and Mennonite people, as is often believed, but arise from gene mutations that were carried from Europe only 300 years ago. The same mutations continue to be expressed elsewhere in Europe, North and South America, and elsewhere in the world. In all human populations genetic disorders masquerade as the familiar conditions called cerebral palsy, autism, epilepsy, SIDS, and, unfortunately, even as child abuse. Current we care for approximately 2000 children with 110 different inherited disorders.

The broader purpose of this essay is to describe how and why I came to work in Lancaster County as a pediatrician and the idea that important frontiers for *translational genetics, genomic medicine, and human biology* are to be found in the everyday work of a physician caring for a patient. I would like to make you believe that my work is both interesting and helpful. I also want to make residents, fellows, and other physicians, who may be dissatisfied with their work, aware that many jobs like mine are available. An Amish Community in Indiana intends to hire three physicians and a PhD to care for children and adults with inherited disorders. In North America there are 2.4 million Amish, Mennonite, Brethren, and Hutterite "souls," living in 200 Communities. Each Community would benefit medically and economically from a Clinic similar to ours.

BACKGROUND

One of the results of my move to Lancaster County was that I did not take my Biochemical Genetics Boards at the end of fellowship, which was of no great matter in Lancaster County but was evidence for others that I had thrown away a promising career in that field.

A few years ago I wrote to the Genetics Board to ask if I could take the exams. I was told if I could summarize my experiences as a fellow with patients and in the biochemistry laboratory they would consider my request. This led to an interesting journey through my fellow's notebooks and some reflection about the sources of the knowledge use in my work.

At the time of my Fellowship in Biochemical Genetics we were required keep a notebook with the goal to describe 50 patient encounters over the Clinical Year of Fellowship - consults, clinic patients, and laboratory diagnoses & follow-up.

A handwritten list in my notebook shows that during the first year I wrote 39 in-hospital consults; only 10 of the names in my list have a final-diagnoses written by them. The diagnostic yield of 24% (10/39) for such consults was probably higher than in many medical centers. My other 29 Consults as a fellow in metabolic disease were for such common reasons as seizures, vomiting/diarrhea with acidosis, liver disease, weakness or hypotonia, ketosis, hypoglycemia, and our metabolic "screens" were uninformative.

Remarkably, 3 of these 10 cases with a diagnosis I have followed for 21 years at my Clinic in Lancaster County. One is a young man with isovaleric academia who presented as an ill neonate, but has not had any other metabolic illnesses. The second was woman with MCADD who presented in infancy with hypoglycemic coma, and survived, 30% then did not. Melissa went on through childhood & school without problems. She became a field hockey star and more recently is a school-teacher. She has had no metabolic illnesses or hospitalizations in 24 years. The third infant is now a 24-year-old Mennonite with MSUD named Nathan, who is the same age as my son. I recently cared for Nathan at Lancaster General following a laparoscopic hernia repair, complicated by a post-operative intra-abdominal hemorrhage, which provoked a long metabolic decompensation. Nathan's hospital course was a reminder of the complexity and fragility of lives of young adults with this disease. During every common illness, after ordinary injuries and otherwise minor surgeries, after child-birth too, their lives hang in the balance of a complex web of biochemistry understood by few physicians.

Five of the 10 patients with diagnoses on my list died - an infant with Pompe disease, a severe variant of Wernig-Hoffman, the first patient described with long chain 3-hydroxy acyl-dehydrogenase deficiency, a girl with mitochondrial complex-1 defect, and a boy with a methionine responsive re-methylation defect, which may have been MTHFR deficiency, who died in early childhood from complications of homocystinemia with renal-vein thrombosis.

In addition to new in-hospital consults, experience with patients came from cases followed by the Metabolic Group at CHOP. I had come from Boston Children's to CHOP to work with Richard Kelley. I began to telephone Richard Kelley about interesting cases in Boston, particularly suspected fatty acid oxidation defects. We diagnosed several patients with MCADD and a boy long chain oxidation defect, which ultimately proved to be one of the earliest cases of VLCAD presenting with cardiomyopathy.

Within the metabolic group at CHOP in 1986 Richard Kelley had the longest list of active patients, 87, with 46 different diagnoses - 13 of these patients were said by him to require major time commitments as a pediatrician and metabolic specialist. The largest grouping of his patients were children with Marfan-like or Ehler's Danlos like connective tissue disorders, which today would be dissected by molecular studies into many different disorders arising from mutations in many genes for

collagens, elastins, or fibrillins. Rik also cared for a collection of patients with neuromuscular problems including Werdnig-Hoffman Disease, congenital metabolic myopathies and cardiomyopathies.

Two brothers with increased 3-methylglutaconic acid in the urine, cyclic neuropenia, and cardiomyopathy would, years later, lead Rik to help discover the multiple etiologies of Barth syndrome and gain fundamental insights into disturbances of myocardial amino acid metabolism that cause life-threatening myocardial pathology. Knowledge that today helps us understand and treat the cardiomyopathy of propionic academia and very-long-chain-acyl dehydrogenase deficiency. (VLCAD)

At CHOP Rik had done organic acids by GC & GC/MS that contributed to the discovery of medium chain acyl-dehydrogenase deficiency (MCADD), the first fatty acid oxidation defect in 1983, then VLCAD, and during my fellowship 3-hydroxy-long chain acyl dehydrogenase deficiency. Our first papers together described the mass spectra of the unusual metabolite 3-hydroxy-octanoate and a method for using GC/MS analysis of octanoate and decanoate extracted from filter paper blood spots to diagnose neonates with MCADD. Rik provided follow-up care for the several of the earliest cases of fatty acid oxidation defects at CHOP. His active case list also included 4 cases of classical homocystinuria and three patients with MSUD.

The first neonate that Rik managed was a Mennonite girl in whom he recognized the need to use free isoleucine and valine supplements to maintain normal plasma concentration ratios between valine/leucine and isoleucine/leucine. This 24-year-old young woman too is cared for at the Clinic For Special Children. Until Richard Kelley introduced this practice most infants followed at CHOP developed anemia, many required transfusions, skin and mucus membrane breakdown, and alopecia. More important, low plasma valine concentrations result in poor transport of valine into the CNS and poor brain growth and development. This idea was later incorporated into ideas about design of the amino acid mixtures used in MSUD hyperalimentation solution and formula. (Morton 2001, Strauss 2010)

In addition to Richard Kelley's patients, by 1987-88 there were 63 patients listed as "actively followed" by the other metabolic specialists in the Division - Stan Segal, Gerald Berry, Mark Yudkoff, Paign Kaplan, and Randy Heidenreich – including 18 patients with MSUD, 10 Mennonite and 8 non-Mennonite.

I was involved in the management of three newborns with MSUD, two of these infants were from the Mennonite population in Lancaster; both have remained my patients for 20 years, one was Nathan, mentioned above, the other was a girl who married two years ago and now has a child, unaffected by her mother's MSUD. Two of our 10 Mennonite children died of cerebral edema during my fellowship, the others I have followed at the Clinic For Special Children since the early 1990s. The next largest patient groups followed at CHOP were isovaleric academia, 9 patients, ornithine carbamyl transferase deficiency, 6 patients, other urea cycle defects, 6, and galactosemia, 6 patients, and 4 patients with methylmalonic academia and 1 case of propionic academia, who was not from the Amish or Mennonite population.

Although our metabolic group listed several "active cases" of storage diseases, these children were typically seen in an outpatient Clinic once each year. I recall seeing children once in Clinic with Pompe disease, cystinosis, and Hurlers disease but I did not have any meaningful clinical or laboratory experiences with this group of disorders.

In 1986 & 87 the metabolic group at CHOP saw about one consult each week, with a diagnostic yield of 20-25% based upon history & exam, plasma amino acids, urine organic acids by GC/MS, and a few selected enzyme assays. No molecular tests were available, the human genome had not been sequenced, the NCBI Data bases did not exist, and McKusick's MIM was printed in 6 volumes. Richard Kelley and five other metabolic physicians followed 150 patients with about 50-55 different genetic disorders. We saw 10 Mennonite children with MSUD but remarkably no other founder-disorders from this nearby population. The division of metabolism saw no Amish children during those years - except for the first patient with glutaric aciduria but it was this single case took my life in a different direction. *In contrast, our small clinic with three physicians in*

Lancaster County, 50 miles west of Philadelphia, currently provides care for approximately 2000 children with 115 different inherited disorders.

ORGANIC ACIDS BY GC VERSUS GC/MS: Urine organic acids by gas chromatography (GC) were also done at CHOP, when specifically ordered. My lectures and consult notes from the time indicated that organic acids should be *considered* if a child had episodic biochemical disturbances like hypoglycemia, anion gap acidosis, lactic acidemias, or hyperammonemias. Handbooks like the Harriet Lane still suggest such findings are an indication for ordering organic acids. The absence of such disturbances in general chemistries was implicitly believed to make an underlying organic academia unlikely, which is not true. Like the wet-lab tests, these handbook-truisms are more often misleading than helpful.

I have cared for infants with glutaric aciduria type 1 that suffer complete destruction of the basal ganglia while serum the anion-gap remains completely normal. Infants who are ill with MSUD are usually alkalotic, not acidotic. Infants with propionic academia and MSUD will when ill sometimes have a positive test for urine ketones, but between illness they usually do not have ketonuria. Using the presence or absence of an anion gap or ketones or hypoglycemia to decide which patients with dystonia should be tested for GA1, propionic academia, or methylmalonic academia will result in most of these patients remaining undiagnosed.

During my fellowship years urine organic acids were done with wide-bore, hand-packed column on a Varian GC with a flame ionization detector, not a mass spectrometer. Tanaka's methylene unit calculations were used to identify compounds. Few of the specimens that were run on the GC were later run through a gas chromatography/mass-spectrometry to confirm the identity of a potentially interesting metabolite. The mass spectrometer we used was an old Finnegan System that filled a room and had computer hard discs the size of floor-buffers, but could only store data from a few GC/MS runs.

My log lists 13 cases called Organic Acidurias that were "diagnosed" in 1986-87.

Only 4 of these were actual diagnoses: 1 case of MCADD, 1 isovaleric acidemia, 1 propionic acidemia, and 1 beta-methylcrotonylglycinuria all four of which would today with be found with newborn screening using MS/MS.

The 9 other so-called *diagnoses* listed in my log were non-specific biochemical abnormalities including 1 neonate with a high urine lactate, 5 cases with high 2-ketoglutaric acid in urine, 2 cases were siblings with increase concentrations of urine methyl-gluconic acid, which would many years later be confirmed by Richard Kelley as a variant of Barth syndrome, and 2 cases of dicarboxylic acidurias, which were in siblings who were known to have Wernig-Hoffman disease.

Gas chromatography, without mass spectrometry, was used at CHOP to *monitor treatment* of patients MCADD, isovaleric acidemia, or other organic acidemias. We all *assumed* a meaningful relationship establish existed between urine or plasma metabolites and metabolic intoxication and that treatment involved control of the dietary intake of protein and fats, and the prevention of catabolism. We collected urine and plasma routinely, and kept the GC lab busy generating data, dictated reports suggesting that illness made selected metabolites go up and therapy made these go down, but such data were seldom studied critically, discussed at department meetings, or reported in publications. In retrospect, I can report that these assumptions were wrong. The urine GC/MS studies did not guide therapy or predict metabolic illness for the glutaric acidurias, propionic academia, MCADD, most other organic acidemias.

The majority of urine organic acids analyzed by GC were to *rule-out* inherited metabolic disorders. Most were done on specimens from outside CHOP and >99% were normal or had minor, non-diagnostic, findings such as a high adipic acid, ketonuria, or lactic aciduria.

From my perspective today, what is most interesting about the organic acid results from our metabolic lab in 1986-1988 was the *absence* of positive tests for disorders that Ed Naylor's MS/MS screening program would 1993-94 discover to be

common in the Plain populations. The lab at CHOP was the only lab in Pennsylvania doing organic acids screens by the wet-lab, GC & GC/MS. There are five common organic acidemias found in the Amish & Mennonite populations of PA: Propionic academia, 3-methylcrotonylglycinuria, MCADD, Glutaric Aciduria Type 1, and Glutaric Aciduria Type 3. Collectively these are much more common than MSUD, yet none of these disorders of the Plain populations had been recognized by the metabolic screening lab at CHOP before the summer of 1988. If today I went through the *wet-lab* dictations from 1986-1988, I would find many names of my current Amish & Mennonite patients with these organic acidemias, “screened for metabolic disorders” and reported have normal *urine organic acid screens*.

In 1994 Ed Naylor’s MS/MS newborn screening program found the first cases of propionic academia and 3-methylcrotonylglycinuria, which are both common in Amish & Mennonite people. We now know that propionic academia arises from the same mutation in the Amish & Mennonite populations (PCCB 1606A>g, Asn536Asp) and is found throughout Pennsylvania, Maryland, and the Midwest.

I found the first Amish cases of 3-methyl-crotonylglycinuria in 1990 in two asymptomatic children, but Naylor’s screening showed the disorder was common in both populations. In 1998 after coming to the Clinic as lab director, Erik Puffenberger found that the mutation 3-methyl-crotonyl-CoA carboxylase is different in the Mennonites (MCCC2 518insT) and Amish (MCCC2 295 G>C, Glu99Gln). Both variants are associated with carnitine low plasma carnitine levels, but none of these patients has even been symptomatic.

I diagnosed the first Mennonite case of MCADD (985A>g) in 1993 in a 14-year-old girl who was being followed at Hershey Medical Center for “ketotic hypoglycemia and cyclic vomiting.” Her infant sister had died in the Lancaster General Hospital emergency room in 1976 and was reported to have a fatty liver, and had been given a pathologic diagnosis of “Reye Syndrome.”

Naylor’s program subsequently showed the MCADD is found in the Mennonite population at about the same frequency as the general population. MCADD is absent from the Amish population, with the exception of a single case of a child homozygous for the T>C199 variant, which probably not a disease. This child has normal ketogenesis during illnesses but she does produce medium chain carnitine esters. Although MCADD was described at CHOP in 1983, none of the cases in the original reports were from the Mennonite population.

The other two organic acidemias that are common in the Amish population of Pennsylvania are Glutaric Acidurias. The first case of the Amish variant of GA1 (GCDH 1262C>t, Ala421Val) was found, by an observant lab tech named Jim Coulter, and some luck, but chance favors the prepared mind, or the interested fellow.

THE GLUTARIC ACIDURIAS

As part of my fellowship research with Richard Kelley, I was routinely using the GC/MS to study the fatty acids and 3-OH fatty acids extracted from culture media of fibroblasts with fatty acid oxidation defects, and whole blood filter paper specimens of infants and children with MCADD. (ref1,2) In June 1988 Jim Coulter showed me a chromatogram and a specimen and said, “Why don’t you run GC/MS on this to see if this is a peak of glutaric acid; that is more than I usually see.” Peak was glutaric acid, about 100 mg/gram creatinine, but that day I did not find the pathologic metabolite 3-hydroxy glutaric acid by GC/MS in this the first urine sample from an Amish patient. As fate would have it, Mike Bennett had just come to CHOP from England where he had done biochemical testing for a Pakistani child with the disorder that was ultimately called GA3, which was thought to be a benign biochemical condition arising from a peroxisomal oxidase defect. (ref) Mike and I discussed the GC/MS finding and expected this would be GA3 or a mildly increased glutaric acid excretion because of diet or medication.

The key to the diagnosis of GA1 was not this specimen, but a conversation with a developmental pediatrician who was director of the Elizabethtown Children’s Hospital for children with cerebral palsy. Dr. Charles Nicter had sent the specimen on this Amish boy because of the history of normal early development ended by the acute onset of dystonia during an intercurrent

illness. Mike Bennett and I drove to Elizabethtown to see the boy. At that time GA1 & GA3 were considered very rare disorders; no one at CHOP had seen either condition, which means we had repeatedly missed the diagnosis. We believed that either this was a patient with GA3 who had developed acute dystonia. Or, we thought this was an example of GA1 without 3-OHGA, and surprisingly low urine glutaric acid excretion, which suggested that if this was GA1 then the Amish variant may be a “partial defect and easily treatable.” In retrospect, Mike and I were wrong about almost everything, and, after 23 years fundamental questions about the biochemistry of GA1 & GA3 remain unanswered. (ref 2007)

On June 19, 1988 I drove to Lancaster County to visit the Lapp family at their farm, to learn more about the disease, re-examine Danny and collect samples from him while on a low protein diet and riboflavin. I had suggested at Elizabethtown Hospital that because he had a “benign biochemical phenotype” that he may respond biochemically. Of course I was wrong, both about the “benign phenotype” and the dystonia. On that trip I learned the names of more Amish families who had children similar to Danny; I began to make trips to Lancaster County to collect urine. By September of 1988 all the major family names were represented in the GA1 pedigree. The disease followed a recessive pattern; many families, like Amos Miller’s, had more than one disabled child. Most case histories were similar to the first patient. In 17 of 20 cases a period of normal development ended with the abrupt onset of generalized dystonia, usually during an infectious illness, and always before 2 years of age. Urine specimens from these cases contained widely varying concentrations of glutaric acid, and using GC/MS I found small amounts of 3-OH-GA in other specimens from the first case, and in Amish children from other families. Lorne Seargeant measured the glutaryl-CoA dehydrogenase activity on lymphocytes and showed the enzyme was absent. By September of 1988 it was apparent that GA1 was common in the Amish Community, all major family names were represented in the pedigree and I could estimate a carrier rate and disease prevalence of at least 1/400 newborns, which was similar to the prevalence of MSUD in the Mennonites of Lancaster County. The birth rate within the community was 1000/year, 2-3 new cases per year. GA1 was common, brain injury was abrupt, irreversible, but appeared to be age dependent. And, most important, even the cases with dystonia appeared to be stable after age 2. The oldest patient I found was a man in his 20s who walked with canes, had developed dystonia at the age of 6 months, but had eventually learned to walk and had stable neurological function, undiagnosed and untreated had no further episodes of illness. He too was a patient of Chuck Nicter’s at the Elizabethtown Hospital. Most important, 1 case out of 20 that I found that summer was a healthy 14 year old Amish girl, who had the same biochemical phenotype as her severely disabled sister, but she had escaped basal ganglial injury.

The complexity of the biochemistry of the glutaric acidurias of the Amish only became apparent in 1990 after my wife, Richard Kelley and I established the Clinic For Special Children and I set-up a lab with GC/MS to monitor therapy of GA1 and test asymptomatic newborns. Within the first 6 months I learned that GA3 was in fact also present in the Amish population; all the specimens I had collected over the summer of 1988 were GA1 specimens from families with disabled children. But once testing of asymptomatic newborns began, I found several newborns with high glutaric acid levels, low 3-OH GA, an absence of glutaryl-carnitine in blood, normal GCDH activity, and an absence of neurological disease. These infants had multiple healthy siblings with the same urine and blood metabolite patterns. I called this condition GA3, assuming these patients had a peroxisomal oxidase defect similar to the Pakistani child Mike Bennett had told me about, and that this was a benign biochemical trait. Fortunately, after some initial confusion, it became apparent that newborns with GA1 could readily be distinguished from GA3. Although older Amish patients with GA1 have relatively low concentration of GA & 3-OH-GA in urine and blood, affected neonates secrete large amounts of pathological metabolites – GA, 3-OH-GA, and glutaryl-carnitine – GA1 and GA3 can be distinguished readily biochemically. we now know GA3 is more common than GA1. With a carrier frequency of 1/6 people GA3 is probably the most common recessive disorder in the population and is now known to arise from a mutation in a mitochondrial acyl-transferase or glutaryl-CoA synthetase (C7orf10 895C>T, Arg299Trp). As reported in our paper published in 2008, 20 years after the first Amish case with glutaric aciduria was found, *Glutaric Aciduria, Type 3: Genetic Mapping to*

Chromosome 7 and Identification of Mutations in C7orf10, Mike Bennett's original patient also has a terminating mutation in this gene. The discovery of this gene was done as a science project by Eric Sherman, a 15-year-old high school student. The Gene mutation was mapped using an Affymetrix 10,000 array to do homozygosity mapping with three Amish children who had been found to have the GA3 biochemical phenotype in the early 1990s.

Finally, the clinical and biochemical complexity of the Amish variant of GA1 has undermined many of my common assumptions about the relationship between disease and biochemistry. My prediction that blood or urine concentrations of glutaric or 3 OH-glutaric concentrations would correlate with the degree of illness or risk of basal ganglial injury was wrong. This was the conclusion after hundreds of blood and urine samples collected after changes in diet, during catabolic illnesses, and at the time of acute degeneration of the basal ganglia. At the time of injury patient are not academic or ketotic or hyperammonemic or hypoglycemic by the usual definition of hypoglycemia. Impending striatal necrosis is associated with an usual form of wakefulness with arrested activity, and sometimes with seizure activity. This stage of brain intoxication responds immediately to a high rate of iv, or enteral, glucose infusion, but not to anti-convulsants or iv boluses of carnitine. Basal ganglial stroke can be prevented by iv glucose infusion, but this protection can not be explained by measurable changes in glutaric acid or its metabolites. Measurements of the fraction excretion of glutaric acid show that the acid is often secreted by the kidney tubule, clearance relative to creatinine exceeds 1, but this secretion is not increased by oral or iv glucose or carnitine, nor by increasing the renal clearance of Na nor by increasing the pH of the urine. The urine typically contains low concentrations of 3-OH-GA, which co-elutes with 2-OH-GA and can only be found, and quantified, by selected ion monitoring. Basal ganglial injury does not correlate with urine, blood or CSF GA or 3-OG-GA levels. In fact, over the years it became apparent that patients with low concentrations of GA and 3-OH-GA in blood and urine were probably at higher risk for basal ganglial degeneration than so-called *high-excreters*. Amish infants have very high concentration of GA-3OH-GA and gluaryl-carnitine in cord blood, amniotic fluid, infant blood and urine, but if fed regularly or given iv glucose show no signs of metabolic illness or encephalopathy. Over time these same infants convert to a so-called, "low excreter." The single case that provided inescapable evidence that my original ideas about metabolic intoxication of GA1 occurred in 1994. The infant was the first Amish child diagnosed by Naylor's screening. I confirmed the diagnosis by GC/MS; his plasma and urine levels of GA & 3-OH-GA were very high. By the time this infant presented with acute striatal necrosis 8 months of age, associated with an infection with RSV, his urine and blood levels had become too low for Jim Couter's old GC; a urine specimen in CHOP's lab at the time of his brain injury would have been called "normal." Erik Puffenberger in 1999 showed this boy to be a compound heterozygote for mutations in GCDH. His maternal great grandfather had been a Cherokee Indian and had probably introduced this mutation into the population, he remains the only Amish case who is not homozygous for the Ala421Val mutation. Low excreters can be converted to high excreters by giving them phenobarbitol. After testing the urine of more than 2000 Amish newborns and running thousands of blood and urine samples from known cases, Amish and non-Amish, by GC/MS with an accurate isotope dilution method, well and ill, injured and non-injured, it was painfully apparent that my assumption that the natural history of the disorder would be explained by such biochemistries was wrong. New insight into the biochemical basis of GA1 came from neuropathologic studies of Christopher Funk and colleagues in 2005 who reported that the brains of 4 children and a 40 year-old adult, from the Cree Indians in Canada and who were low excreters of GA & 3-OH-GA, contained concentrations of GA 150-300 fold higher than blood. Glutaric acid was being formed, and was trapped in brain parenchyma, probably in mitochondria. GA1 & 3-OH-GA were not being formed in peripheral tissue, secreted into blood, to then diffuse or be transported into brain, as my early model based upon toxicology assumed. The report raised as many questions as it answered: all regions of the brain contained high concentrations of GA1 & 3-OH-GA, not just the basal ganglia, and in the brain of the 7 year-old too, not just the patients 18 months or younger. Also of interest, the basal ganglia was histologically abnormal, but the frontal cortex and temporal poles, often reported on CTs & MRIs

to be atrophied were histologically normal. The brains of all cases were remarkably heavy, about 25% heavier than aged matched controls. The GA1 brain large, hypertrophied, not atrophied. What is the substance of this increased mass?

Amino acid analysis by a Beckman ion exchange analyzer was also commonly used to rule out a metabolic causes of general pediatric problems mental retardation, seizures, hypotonia, or low serum bicarbonate and mildly increased plasma ammonias in an ill neonate or child. The diagnostic yield was low. In the years I was at CHOP we did not diagnose, or monitor, any cases of PKU. There were 2 new cases of MSUD diagnosed and managed during 1986-87, and 16 additional cases were managed by the Division - 10/18 were Mennonite, 8/18 were non-Mennonite. Branched chain amino acid analysis was used to monitor dietary therapy, but was not used to monitor management during intercurrent illnesses. In fact, we knew little about how to control the effects of common illnesses and injuries upon metabolism in patients with MSUD - during my second year at CHOP 2 of 10 Mennonite children died of cerebral edema during such illnesses; 14/36 Mennonite children had died of brain edema between 1966 and 1987.

My notebook lists only 39 consults in 1986-1988 to rule out metabolic disease as a cause of vomiting and ketosis, seizures, muscle weakness or hypotonia, mental retardation, and failure to thrive. As indicated above, the yield of biochemical diagnosis was low. One child with myopathy and cardiomyopathy was ultimately shown to have long chain 3 hydroxy acyl dehydrogenase deficiency. Another similar case had lactic acidemia and likely had a complex-I mitochondrial disorder. Two consults for weakness prove to be an infantile Pompe's and Wernig-Hoffman disease. Several cases of hypotonia were signed out as Marfan-like connective tissue disorders. We did not find a metabolic cause of any seizure disorder, but this was one of the more common reasons for a metabolic consult.

I could make a list of 150 patients from my fellowship days representing various experiences with In-Hospital Consults, Clinic Patients, and Laboratory, but after 20 years I am doubtful that this effort would in any way qualify me to take Board Exams in Genetics.

PROGRESS IN THE PREVENTION OF DISABILITY CAUSED BY GA1

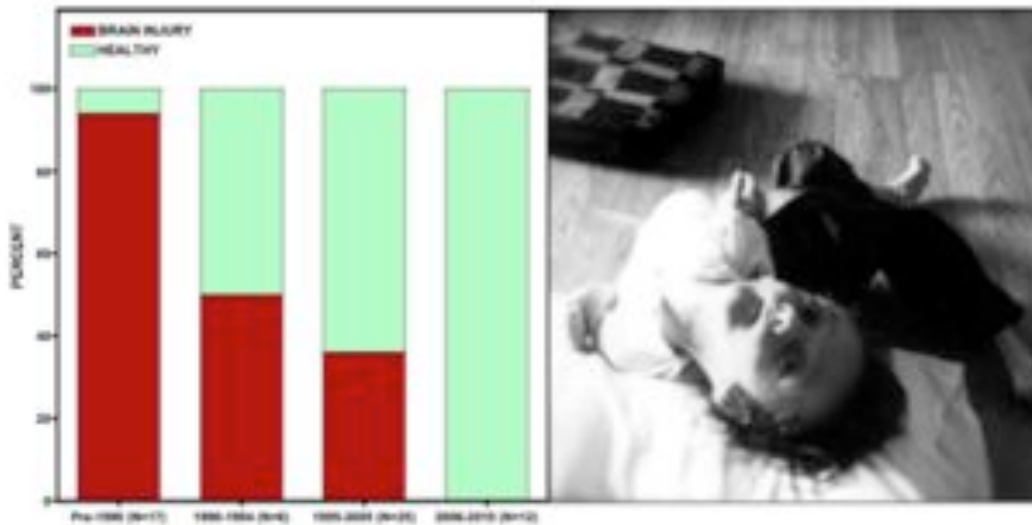


Figure 1. Before 1989, Amish children with glutaryl-CoA dehydrogenase deficiency (GA1) were fully disabled by their second birthday and often misdiagnosed with cerebral palsy. The first genetic study at the Clinic for Special Children identified GA1 as the cause of "Amish cerebral palsy" in 16 children. Beginning in 1989, the Clinic offered on-site diagnostic screening, comprehensive pediatric follow-up care, and inpatient management for children with GA1. The incidence of brain injury decreased from 94% to 36% by 1990. There have been no brain injuries among our 12 GA1 patients born after 2006.

SUBMITTED JUNE 2011, 23 YEARS AFTER MY FIRST VISIT TO LANCASTER COUNTY TO SE AN AMISH CHILD WITH GLUTARIC ACIDURIA NAMED DANNY

Arginine fortification coupled to lysine restriction improves neurological outcome in children with glutaryl-CoA dehydrogenase deficiency

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ABSTRACT

Striatal degeneration from glutaryl-CoA dehydrogenase deficiency (glutaric aciduria type 1, GA1) is associated with the formation and entrapment of glutaryl-CoA and its derivatives within brain, which depends on cerebral lysine influx. In 2006 we designed a study formula with a mixture of free amino acids pattern after human milk but devoid of lysine and with reduced tryptophan, and that was enriched with arginine. The formula provided amino acids to support protein synthesis and kreb cycle anaplerosis, prevent systemic tryptophan deficiency, and provide sufficient arginine to selectively block lysine absorption and limit its transport across cerebral endothelia and thereby limit glutaryl-CoA production by brain. Natural protein (1 – 1.25 gm/kg-day) provided lysine 65-85

mg/kg-day. Between 2006 and present, we treated twelve at-risk infants with study formula (LYSx group) Clinical and biochemical outcomes were compared to 25 GA1 patients (PROx group) treated between 1995 and 2005 with natural protein restriction (dietary lysine/arginine ratio of 1.3 ± 0.4 mg:mg). Arginine fortification to achieve a mean dietary lysine/arginine ratio of 0.7 ± 0.2 mg:mg was neuroprotective. All 12 LYSx patients are physically and neurologically healthy after 28 aggregate patient-years of follow up (current ages 28 ± 21 months). This represents a 36% reduction of neurological risk (95% confidence interval 14 – 52%, $p = 0.018$) that we can attribute to altered amino acid intake. Lysine ingestion was similar between groups, but 2-fold higher arginine intake by LYSx patients was associated with 50% lower plasma lysine, 3-fold lower plasma lysine/arginine concentration ratio, 42% lower mean cerebral lysine influx, 54% higher cerebral arginine influx. Increased intake of isoleucine, valine, threonine, and methionine, all anaplerotic precursors to succinyl-CoA, resulted from increased plasma levels of each amino acid and 22%, 5%, 26%, & 26% increases in cerebral influx respectively. 50% less 3-hydroxyglutarate excretion, and a 3-fold lower hospitalization rate (0.8 versus 2.3 hospitalizations per patient per year). We made several other notable observations: 1) calculated cerebral lysine influx was highest during the perinatal period, explaining the potential for antenatal brain pathology, high organic acid excretion in neonates, and “silent” brain injury during antenatal or perinatal life; 2) large increases in dietary arginine and other free amino acids only produced modest plasma increases, reflecting increased metabolic consumption of amino acids; 3) the relationship between arginine fortification and plasma lysine indicates transport competition at both cerebrovascular and gastrointestinal barriers, suggesting their co-administration is key to efficacy; 4) plasma arginine levels decreased 50% during illness, which may acutely increase cerebral glutaryl-CoA loading and neurological risk; 5) acute arginine depletion was not corrected by dextrose infusion, but could be buffered by an enteral ‘Sick-Day’ formula with lysine/arginine ratio of 0.15 – 0.20 mg:mg; and 6) 70% of lysine ingested by GA1 patients cannot be accounted for by protein accretion, insensible losses, or excretion of metabolic derivatives, implicating a GCDH-independent route for lysine oxidation *in vivo*. Finally, we observe that brain only utilizes approximately 1% of imported lysine for net protein accretion, lysine ingestion by GA1 patients exceeds the demand for somatic growth, and the safe limit of arginine ingestion is well above doses used here. Thus it may be possible to move striatal neurons further away from the necrotic threshold by reducing the dietary lysine/arginine ratio even more, but this should be approached with appropriate caution in growing children.

ADDITIONAL BACKGROUND

Children with glutaryl-CoA dehydrogenase deficiency (GCDH; glutaric aciduria type 1, GA1) can suffer selective striatal degeneration that presents as insidious motor delay or acute motor regression during infancy. Striatal lesions cause movement disorders that are notoriously difficult to treat. Generalized dystonia results in chronic complications such as pain, dysphagia, aspiration, laryngospasm, scoliosis, and restrictive lung disease and often culminates in untimely death.

When the Clinic for Special Children was founded in 1989, management of GA1 focused on presymptomatic detection, natural protein restriction (1.0-1.2 g/kg•day), and sustained oral carbohydrate intake during illnesses, or high rates of glucose infusion above the basal metabolic rate, to suppress of catabolism,

prevention of acidemia with polycitra and bicarbonate, and intravenous hydration with rational to increase clearance of glutaric acid and 3-hydroxy-glutaric acid, weak acids, through the kidney. This was a toxicologist's model of metabolic intoxication. Among our Amish patients a combination of newborn screening, earlier diagnosis and treatment, including in-hospital management of illnesses, reduced brain injury from 94% to 36% between 1990 and 2005. In the early 2000s the toxicologist model was partially displaced by an *Excitatory Toxin Model* of the basal ganglial injury. A model that led to trials with prophylactic anticonvulsants, pharmacologic doses of cofactors and anti-oxidants, including riboflavin, antioxidants vitamin C, CoQ, lipoic acid, pantothenate and carnitine. These additions to therapy conferred no apparent neuroprotective advantage and so were abandoned. (Strauss 2003B) Although population-based studies established the importance of newborn screening for successful treatment of GA1, there remained uncertainties about optimal treatment of asymptomatic infants. Outcome data from multiple centers in Europe provided evidence for the value of dietary lysine restriction combined with metabolic formulas rich in free amino acid mixtures, but, excellent outcomes had been achieved without these expensive and unpalatable formulas. Moreover, because dietary lysine restriction and L-carnitine were almost always used in combination, it was impossible to determine their relative importance to the therapy. Whatever treatment was adopted at a particular center, investigators agreed that urine, serum and CSF studies of glutaric acid and 3-hydroxyglutaric acid were not useful measures of either metabolic control or neurological risk. The information that fundamentally changed our understanding GA1 and changed our approach to treatment were the autopsy studies of Funk et al which suggested GA1 was *cerebral* organic acid intoxication syndrome - Glutaric acid and other metabolites were produced and trapped *within the brain*. The importance of this information was first discussed by Kevin Strauss in 2005 after he reviewed the manuscript for Funk et. al: *Glutaric aciduria type 1: a clinician's view of progress Brain 128 (2005) 697-699. (C.B. Funk, A.N. Prasad, P. Frosk, S. Sauer, S. Kolker, C.R. Greenberg, M.R. Del Bigio, Neuropathological, biochemical and molecular findings in a glutaric acidemia type 1 cohort Brain 128 (2005) 711-722.)*

Humans with GA1 normally express GCDH transcripts in brain. Glutaryl-CoA Dehydrogenase degrades glutaric acid within mitochondria and in its absence glutarate accumulates to concentrations of 500-2000 $\mu\text{mol/kg}$ wet weight, two to three orders of magnitude higher than plasma and cerebrospinal fluid. Based on these data, in 2005 we first posited that GCDH-deficient human brain produced the bulk of glutaryl-CoA and its derivatives from imported lysine. An intercompartmental model of lysine flux suggested that the growing human brain could produce as much as 5,000 to 12,000 μmoles of glutaryl-CoA per kg of tissue from lysine each day. Subsequent animal experiments verified the idea that glutaryl-CoA and its derivatives, once produced within the brain, become trapped due to inadequate cerebral systems for dicarboxylic acid export and low capacity to form carnitine and glycine conjugates. GA3

Based on this line of reasoning, in 2006 we designed a study formula to selectively restrict *cerebral* lysine uptake by exploiting competition between lysine and arginine at the blood-brain barrier (Fig. 1). These amino acids share a common cerebrovascular cationic transporter (γ^+ system) with estimated *in situ* K_m values of 70 μM (lysine), 56 μM (arginine), and 109 μM (ornithine). Thus arginine, within physiological meaningful concentration ranges (reference range $90 \pm 32 \mu\text{M}$), can modulate cerebral lysine uptake. Here we provide a detailed analysis of the clinical and

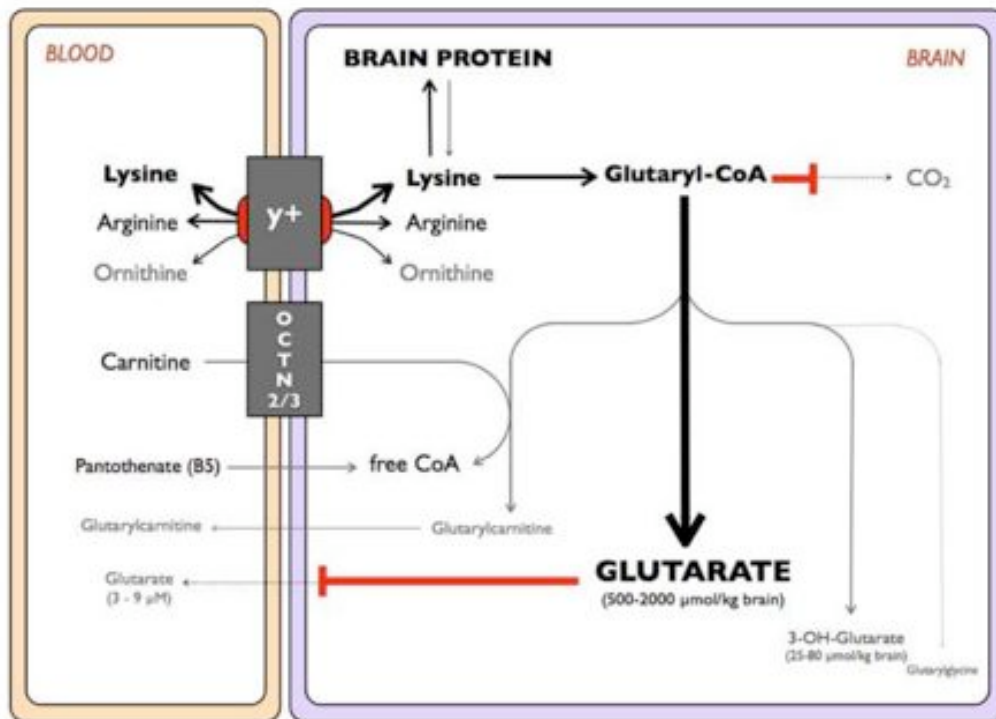
biochemical effects of lysine-restriction coupled to selective arginine fortification, and show that this strategy can in principle reduce brain lysine uptake by 40 - 50% while allowing normal brain growth and development. Twelve consecutive GA1 patients treated with study formula over 28 aggregate patient-years are free of brain injury, suggesting that this treatment may provide a therapeutic advantage over protein-restriction among our patient population.

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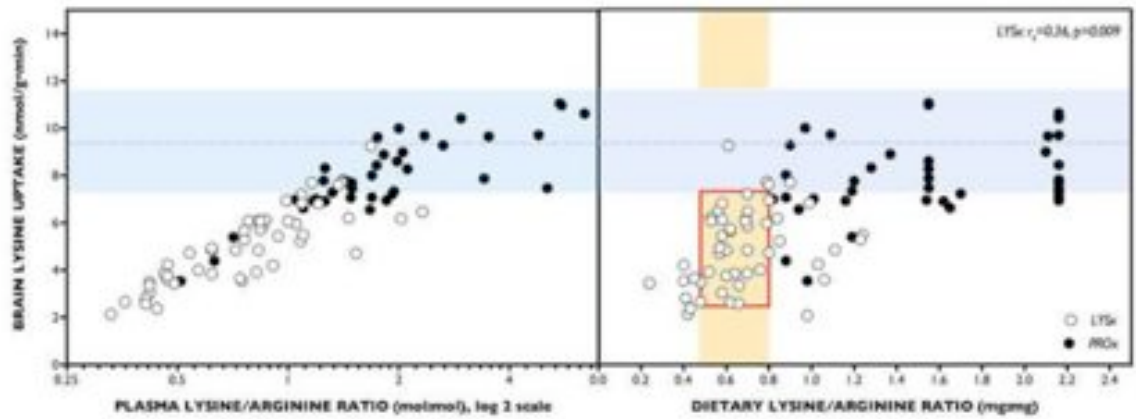
J. Stoll, K.C. Wadhvani, Q.R. Smith, Identification of the cationic amino acid transporter (System y+) of the rat blood-brain barrier J Neurochem 60 (1993) 1956-1959.

Figure 1
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THE NEUROPROTECTIVE EFFECT OF DECREASED LYSINE UPTAKE INTO THE CNS, AS SUGGESTED BY THE Lys/Arg RATIO AND ESTIMATES OF DECREASED LYSINE TRANSPORT RATES USING THE Y+ TRANSPORT EQUATIONS, MAY BE DIRECTLY RELATED TO A REDUCTION OF THE BURDEN OF BRAIN GLUTARYL-CoA.

Figure 5
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CARBOHYDRATE AND AMINO ACID ANAPLEROSIS IN THE BRAIN MAY ALSO CONTRIBUTE TO THE NEUROPROTECTIVE EFFECT OF THE NEW FORMULA.

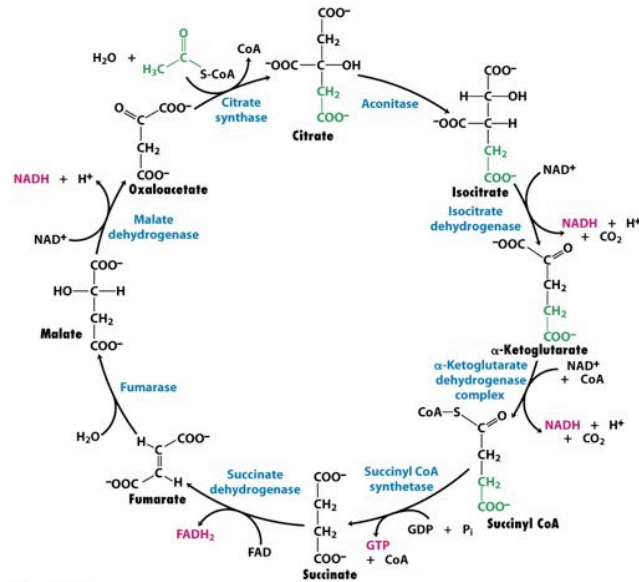
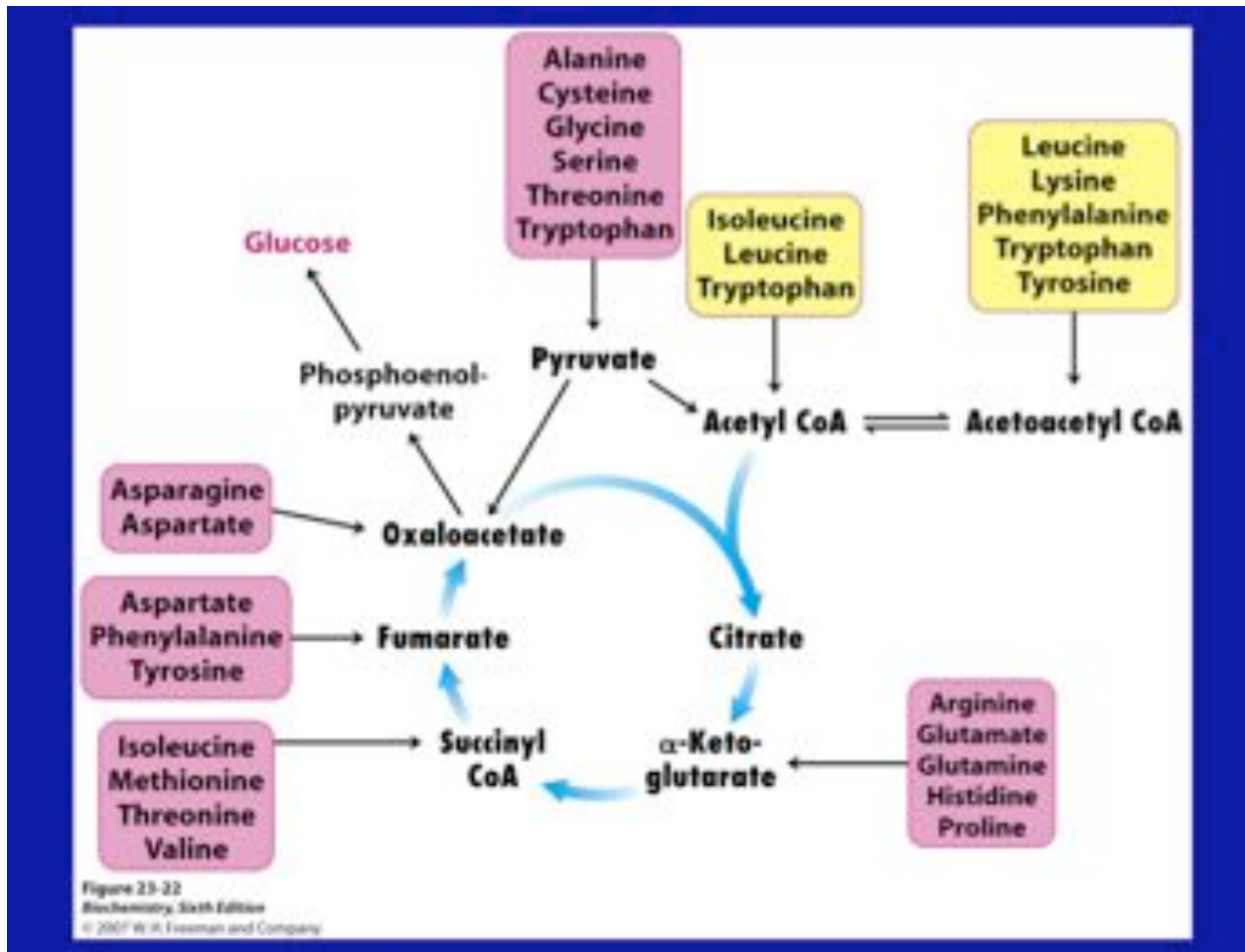


Figure 17-15
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The degradation pathway for lysine enters the mitochondria with the transport of 2-keto-adipic acid, which may share a transporter with 2-keto-glutarate. In mitochondria 2-keto-adipic acid may be acted upon by 2-ketoglutaric acid dehydrogenase itself to reduce NAD⁺ and form glutaryl-CoA. Many Amish people have a benign form of glutaric aciduria called Glutaric Aciduria Type 3. They have a defective acyl-transferase or CoA-synthetase that prevents the formation of glutaryl-carnitine, glutaconic acid and 3-hydroxy-carnitine. (Sherman) Individuals with GA3 do secrete abnormally increased amounts of free glutaric acid into urine. Our speculation has been that these individuals will have low intra-cellular, and low intra-cerebral, concentrations of glutaryl-CoA, which prevents the formation of glutaryl-carnitine but also means that glutaryl-CoA does not enter the citric acid cycle and undergo dehydration and hydration to form glutaconic acid, an analogue of fumarate, and 3-OH-glutaric, and an analogue of malate. We speculate that in patients with GA1, who lack glutaryl-CoA-dehydrogenase, glutaryl-CoA itself or one of its metabolites are competitive inhibitors of the citric acid cycle, probably at the site of 2-keto-glutarate dehydrogenase, succinyl-CoA synthetase or succinate dehydrogenase. The lack of pathologic ketosis in patients with GA1 suggests that succinyl-CoA is available for acetoacetate catabolism, which is probably not the case in propionic & methylmalonic acidemias. Apparently glutaryl-CoA mediated inhibition of the citric acid cycle is prevented by the absence of a glutaryl-CoA synthetase. However, observations of a rapid response to glucose infusion and this paper showing improved outcome with substantial free amino acids suggests the effects of glutaryl-CoA can also be overcome by sustained anaplerotic flux through the citric acid cycle – particularly by CHO > pyruvate, acetyl-CoA, and by free amino acids that contribute intermediates to the citric acid pool: arginine, glutamate, and glutamine > 2-ketoglutarate, and isoleucine, valine, threonine and methionine > via propionate to succinyl-CoA.



THE NEW MODEL OF DISEASE, A NEW APPROACH TO TREATMENT OF AN ORGANIC ACIDEMIA

The current understanding the GA1 metabolic syndrome should be restated as a problem of METABOLOMICS. Although this report indicates that the plasma Lys/Arg ratio may be a particularly useful parameter to monitor, this is not an independent variable. Successful treatment of GA1 also depends upon a well designed metabolic formula to provide adequately caloric intake, amino acids, and vitamin cofactors to sustain growth and nutrition. Flux of carbohydrate, pyruvate, and anaplerotic products of selected amino acids in the brain may prove to be particularly important. Many children with GA1 are injured during fasts and catabolic state associated with infectious illnesses. The increase in the Lys/Arg ratio cause by fasting associated decreases in plasma arginine reported in this paper provides new insight into the cause of acute injury, and a new opportunity for therapy. However, hypoglycemia and the decreased anaplerotic flux of citric acid substrates are also likely to be important variables of care.

Beyond the details of biochemistry and metabolism, the cornerstone of successful treatment of GA1 will always be early diagnosis and treatment. The neonatal transition period is an especially interesting time in an infant with GA1. Ideally, at-risk infants should be diagnosed using cord blood and the metabolic disorder should be managed expectantly. Translational medicine in the public health domain requires newborn screening. GA1 cannot be treated unless infants are recognized before basal ganglial degeneration occurs. At the Clinic For Special Children the success of getting infants through the neonatal transition period ultimately depends upon education of parents and midwives to do early diagnosis using cord blood for molecular testing and to at least give oral glucose every 2 hours or, preferably, GA1 formula.

The 1989 model, which led to the first success in preventing basal ganglial degeneration 21 years ago, was a toxicologist's concept focused upon preventing the accumulation of glutaric acid in blood by the prevention of sustained catabolism through nutritional control – sustained-growth in well child without protein intake in excess of that needed to support growth – human milk provides per 100 Calories, 1.6 grams of high quality protein, that is protein relatively rich in essential amino acids – Lysine 66 mg, Arginine 34 mg and 101 mg Leucine. The tolerance of leucine in a newborn with no branched chain keto-acid dehydrogenase activity, classical MSUD, is 50-60 mg/kg-day, which means this is amount of leucine needed to sustain growth and prevent the accumulation of leucine in plasma. However tolerance of leucine changes, decreases, as growth velocity slows at 6-12 months of age – by 18 months of age the tolerance of dietary leucine has decreased to 20-25 mg/kg-day. The GA1-Lys-x study formula provides 69 mg Lysine (1X) and Arginine 149 mg - 4.4 X normal intake – with the consequence of a low ratio of plasma Lys/Arg concentrations, calculated decreased up-take of Lys into the brain and decreased degradation of Lys to glutaric acid and decreased accumulation of glutaryl-CoA within the mitochondria of the brain. Glutaryl-CoA is currently thought to be the problematic metabolite.

Why? How? What is the mechanism of toxicity? Why is the brain selectively affected between birth and 18 months? Glutaryl-CoA concentrations are high in all regions of the brain at all ages, but the putamen is selectively vulnerable in the first year of life. Why? The heart, skeletal muscle, liver, and kidney all have high glutaric acid concentrations and, possibly high glutaryl-CoA, levels but there is little or no apparent mitochondrial toxicity to these organs at any age. Are these organs protected from toxicity by low glutaryl-CoA synthetase activity, active

dicarboxylic transporters, or by high glutaryl-carnitine transferase activity? Does the propensity of these organs to use lipid and branched chain amino acids as a fuel source convey protection, in contrast to the brain, which is both glucose & amino acid dependent. The brain of GA1 children is vulnerable to hypoglycemia. Even short fasts 6-8 hours and modestly decreased levels of blood glucose 60-70 mg/dl may be associated with somnolence, “behavioral arrest,” which responds dramatically to oral or intravenous glucose infusions and increased blood glucose concentrations 110-130 mg/dl. Although differences in carbohydrate intakes between study groups do not explain the improved outcome in the recent study, I have little doubt that the sustained supply of glucose remains an important treatment parameter. However, it is increasingly apparent that in GA1 and propionic acidemia, as in MSUD and MCADD, that control of brain metabolism cannot be reduced to glucose infusion or transport rates. Amino acids play a critical role in citric cycle dependent energy production in all organ systems, regardless of whether the primary fuel is carbohydrate-pyruvate as in brain or lipid-ketone bodies as in heart. Energy production requires sustained anaplerosis of citric acid cycle-intermediates by free amino acids. I think anaplerotic flux into the citric acid cycle, particularly at the succinyl-CoA site, will ultimately prove to be the weakness of protein restriction as a primary approach to lowering brain glutaryl-CoA concentrations, and, an essential element of the success of Lys-x formula. Intakes of the amino acids that supply succinyl-CoA are all markedly increased in the formula as compared to a diet of human milk: ILE mg 146/55 = 2.6 X; VAL 160/56 = 2.9 X; THR 114/45 = 2.5 X; MET 57/13 = 4.4 X; ARG 149/34 = 4.4 X; GLN 342/180 = 2.4 X; HIS 62/36 = 2.5 X; GLU 88/78 = 1.1 X. The blood brain barrier uptake of the LAT1 dependent AA – ILE, VAL, THR, & MET are increased respectively, 22%, 5%, 26%, and 26% and Arginine a precursor for 2-keto-glutaric > succinyl-CoA is increased 56%. These and all other amino acids ultimately contribute to citric acid-cycle anaplerosis and allow normal flux of 2-carbon-units from glucose and lipids through the citric acid-cycle and normal energy production. Goals of therapy for GA1 and other metabolic therapy should not be thought of in terms of a single variable like reducing glutaryl-CoA or Lys/Arg but in terms of sustained energy production during times of normal growth and intercurrent illness. Sustained energy production requires not only organ specific substrate availability as a source of energy but citric acid-cycle anaplerosis from amino acids as well as normal perfusion, O₂ supply, metabolic cofactors, acid-base balance, LAT1, Y⁺, GLU1 transport, and normal CSF production/resorption as well as local blood flow to the basal ganglia to meet changing metabolic demands. What emerges from this broader thinking about this metabolic disorder and its natural history is a conceptual model that is similar to earlier model of a toxicologist, but it's really the Pathophysiologic Model of a

Biochemical Disorder used by Richard Kelley and others before molecular cascades dominated disease models, which not long ago was called in grants SYSTEMS BIOLOGY and more recently the buzz-word is METABOLOMICS.

So what is our conceptual framework for managing GA1 today?

- 1) Diagnosis and treatment off the presymptomatic infant
- 2) Sustained control of metabolism in the well, growing infant and during inter current illnesses.
- 3) The brain is uniquely dependent upon 2-carbon units of carbohydrates and anaplerosis of citric acid cycle intermediates derived from amino acids, particularly those that depended upon the LAT1 and Y+ blood brain barrier transporters and flow through the propionate and 2-ketoglutarate pathways to form succinyl-CoA.
- 4) Glutaric acid is formed within mitochondria by decarboxylation of 2-keto-adidpic, which may depend upon 2-keto-glutarate decarboxylase itself. Some free glutaric acid may be transported out of mitochondria by dicarboxylic acid transporters, however, it appears that free glutaric acid itself is not of much consequence. GA3, which is glutaryl-CoA synthetase deficiency prevents the formation of intra-mitochondria glutaryl-CoA, is a benign disorder. In the Amish population, ironically, this recessive disorder appears to be more common than GA1. (Sherman et al) The degradation of glutaric acid is blocked in glutaryl-CoA dehydrogenase deficiency GA1 but two intermediates of the pathway are formed anyway – glutaconate & 3-hydroxyglutarate as well as carnitine conjugates of all three CoAs.
- 5) It seems likely to me that the complex that acts upon glutaryl-CoA is succinyl-CoA dehydrogenase, a dehydration to form glutaconyl-CoA, fumase to hydrate the 2-3 bond and form 3-OH-GA but malate dehydrogenase cannot form the 3-keto-glutarate, which is the analogue of oxaloacetate. 3-hydroxy-glutarayl-CoA may be a competitive inhibitor of malate dehydrogenase and/or glutaryl-CoA may be a competitive inhibitor of succinyl-CoA dehydrogenase. When these compounds accumulate to high concentrations AND when succinyl-CoA and other kreb intermediates are depleted and glucose flux is low then toxicity of glutaryl-CoA accumulation is expressed. Normally, the flux of glucose and availability of succinyl-CoA is adequate to overcome the inhibitory effects of these pathological CoA esters. However, fasting & illness with the associated decreased rates of glucose & amino acid

transport, changes in local perfusion/oxygenation/further increases in pathological CoA esters/depletion of free CoA/low activity of basal ganglial acyl-transferases may produce critical inhibition of kreb cycle function and energy depletion that activate tissue apoptosis cascades. Although this presents a completely different model of GA1 toxicity, it is understandable that the early interventions did protect some patients. The current model provides many different approaches to limiting the effects of glutaryl-CoA/3-hydroxy-glutaryl-CoA, including the mechanism suggested by this paper to be very important – changing the LYS/ARG ratio and thereby limiting LYS transport into the brain and limiting glutaryl-CoA accumulation.

- 6) What role does L-carnitine have in neuro-protection? Carnitine is made, primarily in liver. The brain does not make carnitine and has low carnitine-acyl-transferase activity as compared to liver, heart & kidney. But, apparently there is a carnitine transporter. My sense is that carnitine is not neuro-protective in a significant way. It may lower glutaryl-CoA by some small percentage in brain. Is probably more important in liver, heart, skeletal muscle & kidney to prevent high mitochondrial concentrations of glutaryl-CoA & 3-OH-G. There is no reason to think carnitine is harmful, other than GI effects, fish-odor, & cost all result from excessive doses.

SUMMARY

In 2006 we designed a study formula to selectively restrict *cerebral* lysine uptake by exploiting competition between lysine and arginine at the blood-brain barrier (Fig. 1). These amino acids share a common cerebrovascular cationic transporter (γ^+ system) with estimated *in situ* K_m values of 70 μ M (lysine), 56 μ M (arginine), and 109 μ M (ornithine). Arginine within physiological meaningful concentration ranges (reference range $90 \pm 32 \mu$ M) can modulate cerebral lysine uptake. Here we provide a detailed analysis of the clinical and biochemical effects of lysine-restriction coupled to fortification with arginine and other anaplerotic amino acids. We have shown that this strategy can in principle reduce brain lysine uptake by 40 - 50% while providing sufficient carbohydrates and amino acids to allow normal brain growth and development in the critical period before 18 months of age. Twelve consecutive GA1 patients treated with study formula over 28 aggregate patient-years are free of brain injury. The authors believe treatment with Glutarade formula and 1 – 1.5 grams/kg-day of natural protein provides a therapeutic advantage over protein-restriction in our patient population.

Amos' Child

PAINTING BY SARAH MCRAE MORTON , Oil on linen 6 X 4 feet



Amos' Child

D. Holmes Morton M.D.

Amish friends, Amos' family, spent Saturday afternoon at our home last summer. Amos asked me about a small telescope on our back porch. I explained that my children and I used it to look at mountains and craters of the moon and the rings of Saturn. Amos didn't know that men had walked on the moon 13 times but seemed neither surprised nor impressed by the fact. He asked, "Have you thought much about why the stars are there? Do you think God made the moon and stars just to look at? What is the moon for?"

Amos & Susie Miller had five special children. They asked many times Why does God give us these children? What are special children for? Modern genetics does not provide an answer for Amos and Susie. The science does not even allow the question. But within cultures that endure for hundreds of years, as the Plain cultures have, there are answers to such questions. For the Amish people beliefs, faith, events, stories, work, histories, the elderly, children, and the stars do have purposes. I believe that if we are to provide adequate care for special children of the Plain people then we must appreciate the place these children have in their families and communities.

I well remember my first visits to the home of Amos.

I could see the rusted wheel chair of a child beside the plow in the barn that I walked past and I thought it likely that I had found the right farm.

Are you Amos Miller? I asked.

Amos said, That's what they say. Who are you?

My name is Holmes Morton. I am a doctor. John and Ida Lapp told me about your children. They are like Daniel. I think I know what causes such children to be crippled.

Amos, You say you are a medical doctor?

Yes. I said.

Amos, we haven't heard of you. Other doctors thought they knew about these children too. Have you ever heard of Dr. McKusick? He was here before. He studied our children at John's Hopkins, spent a lot of money, and couldn't give us an answer.

Yes. I know Dr. McKusick. The disorder I suspect is not one for which he would have tested. I said.

Amos, well, you can come in. My supper is on the table but I am sure my wife will want to talk to you.

Years after my first visit Susie still told others, I was so surprised when Amos let Dr. Morton in the house. Amos can be tough with salesmen, especially, when they come at suppertime. Amos went to the door determined to send him away so we could finish our supper. At first, I just couldn't imagine why Amos let him in.

Amos remembers, Morton talked for more than an hour. He said he thought he knew what happened to John and Ida's Danny and suspected that our children were crippled by the same disease.

He didn't even want money. I could tell by his car he wasn't one of those money doctors. I never saw a doctor drive an old car like that. He didn't even want blood, just some urine from the crippled children. He said there was something in the urine that would explain why our children were crippled.

We didn't believe him. After 18 years with five crippled children. So many doctors, so many hospitals, so many tests without answers, then, a young doctor knocks on our door and offers to do a test for free. He said he was just interested in trying to help such children. How could we believe him?

Susie remembers, when Dr. Morton came back with the test results Amos was not at home. I was ironing. He sat in the rocking chair by the window. He said the test showed what he thought it would. The test gave the explanation about why our children were crippled. I just kept ironing. For a long time I could not say anything but I just shook inside. I thought about our sons who died, first Sylvan, then Levi, and about our other crippled children. I thought about unborn children and the hope that they might now have. I just shook inside. After all these years how could it be? It was so hard to believe because of the way it happened. Yes, I remember the first day Dr. Morton came to our house and how surprised I was when Amos let him in.

Susie told me, the death of Sylvan was especially hard for Amos. He had become Amos' child. Sylvan was 6 months old when he became helpless. It happened so fast. He had a cold. In the morning he had a fever, at noon when I put him down for his nap he seemed normal, but, when I got him up at 3 o'clock he was helpless. He was limp, couldn't hold his head, he could no longer sit, or take a bottle normally. I was so young then and by then Levi was helpless and Elmer had to be cared for too. There was not any thing I could do but ask Amos to take care of Sylvan. He fed him and got up at night for him. Even when Sylvan was small Amos took him to the barn to do the milking, and to the fields on the plow and the hay wagon. Sylvan was Amos' child.

Sylvan died on a Sunday afternoon when we were on our way to visit friends. It happened along the Bartville Road not far from David Fisher's. Amos looked back in the carriage and saw that Sylvan was still. He said "Susie I don't think Sylvan is breathing!" He stopped the carriage in the middle of the road and handed me the reins. I had the baby on one arm and had to hold the horse with the other arm while Amos ran back to pick-up Sylvan. Amos said "He is dead Susie. Sylvan is dead."

We went to the next farm house. They took us in and comforted us. I just did not know what Amos would do without Sylvan. It was almost more than he could bear.

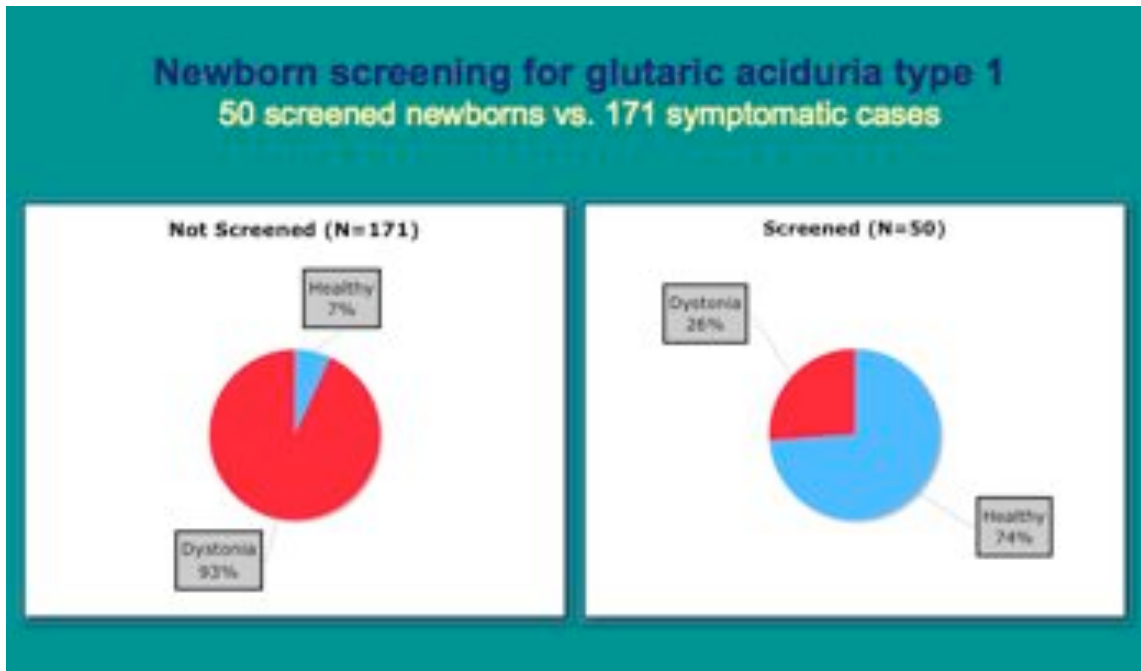
The day after Sylvan died was the great snow storm of 1978. We didn't know if we would be able to bury him but the men worked hard to get the road open to the cemetery and the grave dug. We buried him while snow blew over us and it was very cold.

Not long ago when I drove through that part of the county to make a house call, I stopped at the cemetery where Sylvan is buried. I could imagine the day of his burial - the snow falling, blowing, covering the headstones, covering the fresh earth from the grave, falling into the grave, onto the horses and carriages along the fence. I could see people in black walk past the open coffin. I could see the blue-white face and hands of the dead boy. The snow fell onto his face and hands and did not melt. From time to time as people filed by, Amos would gently use his bare

hand to brush the snow from Sylvan's face. The sound of the earth falling on the pine coffin was softened by snow and by the time the family drove away the grave was filled and hidden under snow.

I thought, "My work began then. Ten years before I came to Amos Miller's door. Even before I was a doctor. The work began with the life and death of an Amish boy who had become his father's child. Amos would have sent me away if it were not for his love of Sylvan, for Susie said, Amos was tough on salesmen who came at suppertime. At first, she was so surprised when he let me come in."

dhm, Strasburg PA April 1994





THE CLINIC FOR SPECIAL CHILDREN AS A PARADIGM OF GENOMIC MEDICINE

The translation of genetic information into patient care begins with a commitment to care for the patient. Frances Peabody in a lecture to Harvard Medical Students in 1927 remarked, *One of the essential qualities of the Clinician is interest in humanity, for the secret of caring for the patient is in caring for the patient.*

Our work at the Clinic for Special Children over the past 22 years has emphasized a commitment to caring for patients who have inherited disorders. The humanitarian need for our work is apparent, but the scientific importance of our daily work should not be underestimated. Many facets of the natural history of genetic disorders only become apparent to physician-scientists who care for individual patients over long periods of time and who are interested in biology, pathophysiology, and the practice of medicine in the most general sense.

The identification of underlying molecular lesions alone usually provides little insight into the phenotypic diversity that we encounter in patients in Lancaster County. The complex neurological syndromes of Glutaric Aciduria, Maple Syrup Urine Disease, and Phenylketonuria arise from single gene mutations, but the complex mechanisms of brain injury and dysfunction are linked to vulnerabilities during critical periods of early brain development. In patients with Glutaric Aciduria episodes of metabolic illness that cause acute striatal necrosis in a 12-month-old infant are well tolerated in the 6 year old and adult. The unbalanced amino acid transport into the brain that leads to mental retardation in the infant with Maple Syrup Urine Disease or Phenylketonuria will cause attention deficit disorder and mood disorders in teenagers and pre-senile dementia in older adults. Single gene disorders give rise to disease states through the disruption of critical biological processes such as metabolic adaptation to fasts and infectious illnesses, cell volume regulation, brain amino acid homeostasis, and postnatal brain growth and development. Scientists who work as physicians and care for many patients with the same genetic

disorder over long periods of time develop a different understanding of genetic disease than scientists who study disease mechanisms in cell cultures in the laboratory. It is often through the daily work of a physician caring for a patient that new opportunities for treatment are realized.

The every day practice of medicine is a frontier of Translational Genetics. The medical care of a child with a genetic disorder is no different than the care of a child with other complex medical problems - diabetes mellitus, depressive illness, rheumatoid arthritis, seizures, and many other chronic troubles come to mind. As with the majority of diseases treated by physicians, there are few perfectly treatable genetic disorders. Nonetheless, improved understanding of disease mechanisms, combined with accessible and effective medical care can often decrease suffering, improve function, reduce dependency upon others, and otherwise limit the effect of disease, genetic and acquired, upon the life of a patient.

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GENERAL:

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