

Pediatric Medicine and the Genetic Disorders of the Amish and Mennonite People of Pennsylvania

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The Clinic for Special Children in Lancaster County, Pennsylvania, is a community-supported, nonprofit pediatric medical practice for Amish and Mennonite children who have genetic disorders. Over a 14-year period, 1988–2002, we have encountered 39 heritable disorders among the Amish and 23 among the Mennonites. We emphasize early recognition and long-term medical care of children with genetic conditions. In the clinic laboratory we perform amino acid analyses by high-performance liquid chromatography (HPLC), organic acid analyses by gas chromatography/mass spectrometry (GC/MS), and molecular diagnoses and carrier tests by polymerase chain reaction (PCR) amplification and sequencing or restriction digestion. Regional hospitals and midwives routinely send whole-blood filter paper neonatal screens for tandem mass spectrometry and other modern analytical methods to detect 14 of the metabolic disorders found in these populations as part of the NeoGen Inc. Supplemental Newborn Screening Program (Pittsburgh, PA). Medical care based on disease pathophysiology reduces morbidity, mortality, and costs for the majority of disorders. Among our patients who are homozygous for the same mutation, differences in disease severity are not unusual. Clinical problems typically arise from the interaction of the underlying genetic disorder with common infections, malnutrition, injuries, and immune dysfunction that act through classical pathophysiological disease mechanisms to influence the natural history of disease. © 2003 Wiley-Liss, Inc.

KEY WORDS: genetic diseases; general pediatric medical care; metabolic diseases; genotype-phenotype correlation

INTRODUCTION

Features of the Population

The manuscript of John A. Hostetler's book *Amish Society* was reviewed in the early 1960's for Johns Hopkins University Press by Victor McKusick

[Hostetler, 1963]. McKusick recognized the importance of the book as a sociological study and realized the potential of Amish populations for the study of human genetics. McKusick's review, and the subsequent publication of *Amish Society* in 1963, marked the beginning of a collaboration between Hostetler and

McKusick that spanned several decades. In 1978 McKusick edited and Johns Hopkins Press published *Medical Genetic Studies of the Amish*. [McKusick, 1978] Table I lists 26 genetic disorders that were described in this publication, which came from many different Amish demes throughout Pennsylvania and the midwest. Puffenberger and Francomano discuss the origins of genetic studies in the Mennonite and Amish populations in accompanying articles [Francomano, 2003; Puffenberger, 2003].

Although these populations are unique in many ways, when compared to the general population of Pennsylvania, the Amish and Mennonites are less distinct than is generally appreciated. In each of the 14 or more generations since the early 1700s, 10–20% of the children have left the Old Order communities [Hostetler, 1993]. Lancaster County church cemeteries, public school rosters, and regional phone books include many Amish and Mennonite family names. Therefore, many so-called Amish alleles

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TABLE I. Metabolic Disorders and Syndromes Found in Medical Genetic Studies of the Amish [McKusick, 1978]

	Inheritance	OMIM	Deme ^b
Metabolic disorders ^a			
<i>Phenylalanine hydroxylase deficiency</i>	AR	261600	IN/PA
<i>Propionyl-CoA carboxylase deficiency</i>	AR	232000	OH/PA
<i>Pyruvate kinase deficiency</i>	AR	266200	PA(B)
Syndromes			
Albinism, oculocutaneous, tyrosinase deficient, type 1B	AR	606952	IN
Spastic paraplegia	AD		PA(L)
Arthrogryposis, dysmelination, craniosynostosis, and cleft palate	AR		
Ataxia-telangiectasia	AR	208900	IN
Brittle hair, intellectual impairment, and decreased fertility	AR		
<i>Byler disease, progressive familial intrahepatic cholestasis</i>	AR	216400	PA(Lw)
Cardiomyopathy, asymmetric septal hypertrophy	AD		IN
Cardiomyopathy, nonobstructive	AD		OH
<i>Cartilage-hair hypoplasia</i>	AR	250250	PA,OH,IN
Charcot-Marie-Tooth syndrome	AR	118200	
Coclear deafness, myopia, and intellectual impairment	AR	221200	PA(L)
Jackson Weiss syndrome	AD	123150	IN
Deafness, congenital	AR		
<i>Ellis-van Creveld dwarfism</i>	AR	22550	PA(L)
Epidermolysis bullosa letalis	AR	226700	OH
<i>Familial manic-depressive illness</i>	AD	136800	PA(L)
Hypothyroidism and muscular hypertrophy			
Limb girdle dystrophy, type 2E		604286	IN/PA
Mast syndrome	AR	248900	OH(H)
<i>McKusick-Kaufman syndrome</i>	AR	236700	OH/PA
<i>Muscular dystrophy, limb-girdle type</i>	AR	253600	IN/PA
Nanophthalmos, familial	AR	251600	NR
Oculocerebral syndrome with hypopigmentation	AR	257800	
Renal-retinal dysplasia	AR	266900	
Troyer syndrome	AR	275900	OH(H)
Weill-Marchesani syndrome	AR	277600	PA(L)

^aDisorders in italics are also entered in Tables II and III.

^bPA(B) is Belleville, PA; PA(L) is Lancaster county, PA; PA(Lw) is Lawrence county, PA, OH(H) is Holmes County, OH.

or Mennonite alleles have diffused out into the general population. The Old Order Amish and Mennonite populations of Pennsylvania are new compared to the time of origin of these gene mutations. All three of the mutations causing phenylketonuria (PKU) in the Old Order Amish and Mennonites are found throughout Europe. The so-called Amish mutation for glutaric aciduria type 1 (GA1) is one of the more common mutations found throughout Europe and the United States [Biery et al., 1996]. Therefore, for most disorders

found within the Amish and Mennonites, the alleles are not unique, but these alleles are at different frequencies than in

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the general population. The gene mutations carried by the founders of the demes of Pennsylvania are not distributed evenly, either within or among the many subpopulations of the state. For 18 of the disorders seen regularly at the clinic, the incidences are high, approximately 1/250 to 1/500 births. Although there is a wide variety of heritable disorders among the Plain people (Table I), the majority of founder disorders are clustered in a few families, and the overall disease prevalence among Plain people is relatively low. Examples

of lower-frequency founder disorders include PKU and medium-chain acyl dehydrogenase deficiency (MCADD), which are probably less common in the Plain populations of Lancaster County than in the general Caucasian population. Some common genetic diseases were apparently excluded by founder sampling and/or genetic drift. For example, cystic fibrosis is not seen in the Plain people of Lancaster County but is found in some other western Pennsylvanian demes. This uneven distribution reflects different founders and a 300-year separation of the various subgroups prior to, and after, settlement in North America. In the Plain people of Pennsylvania, maple syrup urine disease (MSUD) is found only in those of Mennonite descent and GA1 is found only in the Amish populations. Although eight disorders are found in both populations, only Crigler-Najjar disease and propionic acidemia are currently known to arise from the same mutations in both populations. MCADD, 3-methylcrotonylglycinuria [Gibson et al., 1998], and at least two forms of osteogenesis imperfecta are found in both populations but are caused by different mutations in the Amish and Mennonites [Puffenberger, 2003].

MEDICAL CARE AND THE CLINIC FOR SPECIAL CHILDREN

The early genetic studies of the Plain people indicated unique needs for the medical care of people who were geographically and culturally isolated. The Clinic for Special Children was organized to integrate general medical care and modern genetics [Hostetler, 1993; Morton, 1994]. An initial goal of the clinic founders was to diagnose MSUD and GA1 in asymptomatic neonates and prevent the metabolic crises in order to prevent brain degeneration that characterized these conditions. Because common childhood infections often provoke metabolic crisis in children with these disorders, we reasoned that medical care for affected children should be directly provided by individuals who were knowledgeable about the disor-

ders, who had laboratory facilities to diagnose and monitor biochemical control during infectious illnesses, and who were interested in general pediatric practice [Morton et al., 1991; Morton, 1994; Morton et al., 2002b]. Because the early symptoms of these serious disorders are difficult to distinguish from common diseases, we often evaluate patients who turn out not to have a genetic disorder common to the Plain people. Infants who present with failure to thrive and irritability or who become unusually ill during otherwise common infections most often do not have underlying genetic disorders. After the clinic opened in 1989, it was apparent the practice would not be limited to patients with MSUD and GA1. The first ill neonate sent to the clinic to rule out MSUD did have a family history of MSUD, but instead had Hirschsprung disease with bowel obstruction and sepsis. One of the more recently identified infants with MSUD also had a second rare recessive disorder, severe combined immune deficiency. Two of our patients with MSUD have first cousins with MCADD. Before 1988, all Amish children with GA1 were said to have cerebral palsy. Many children with so-called Amish cerebral palsy did have GA1, but others did not, and their evaluations led to the descriptions of troponin T1 myopathy [Johnston et al., 2000], Amish lethal microcephaly [Kelley et al., 2002; Rosenberg et al., 2002; Strauss et al., 2002], and an autosomal recessive Pelizaeus-Merzbacher-like central dysmyelination syndrome [Morton et al., unpublished results]. We recently evaluated a neonate with a sibling with GA1 who fortunately did not have GA1, but unfortunately, as we learned from a neonatal screening test, his hypospadias was a sign of another recessive syndrome, 3- β hydroxysteroid dehydrogenase deficiency. Another family has three children with GA1 and a fourth with PKU. Over the years, failure to thrive workups have led to the diagnoses of Byler disease, three new disorders of bile salt transport [Morton et al., 2000], Bartter syndrome, aldosterone deficiency [Mitsuuchi et al., 1993], and primary growth hormone defi-

ciency [Morton et al., 2002a]. Clearly, the burden of genetic disease is high and the need for specialized and comprehensive care is essential to the health of the community. In the last 14 years we

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have cared for patients from the Amish and Mennonite populations with more than 60 different single-gene disorders, as shown in Tables II and III. These tables summarize a clinical experience with 400–500 patients since 1988. Tables II and III are not complete lists of genetic disorders found in the Amish and Mennonite populations of Pennsylvania. However, we have seen patients from other settlements in central and northwestern Pennsylvania where different founder genes presumably explain the prevalence of different sets of genetic disorders.

CULTURAL AND ECONOMIC BACKGROUND OF THE CLINIC

The Clinic for Special Children was registered in Pennsylvania as an independent nonprofit organization in 1989. The original board of directors included three of the authors of this paper (D.H.M., C.S.M., R.I.K.) and the late Dr. John A. Hostetler, a descendant of Amish parents and a sociologist of rural culture. In 1990, several parents of children with genetic disorders from the Amish and Mennonite communities joined the board. Both communities were aware of significant numbers of children with MSUD, GA1, and other genetic disorders. The goal of these parents was for the clinic to provide comprehensive medical care for children with complex and unstable disorders. The Clinic was viewed as a way to

TABLE II. Genetic Disorders of Pennsylvania Amish

	Illness during infancy	History and exam	Neo gen or PA screen	Serum amino acid analysis	Urine or (serum) organic acids by GC/MS	Amino acids and acyl carnitines by MS/MS	Diagnosed by mutation detection ^a	Clinical notes
Metabolic disorders								
Adenosine deaminase deficiency	+							Milroy Amish. Severe combined immune deficiency. Lethal w/t bone marrow transplant
Adrenal hyperplasia II 3 β -hydroxysteroid dehydrogenase def.	+		+		+		+	Neonatal hyponatremia/hyperkalemia. Males have ambiguous genitalia but severity variable
Congenital growth hormone deficiency	+							Poor growth in infancy with anorexia and low IGF-1 and GH
Corticosterone methyl oxidase II def. Aldosterone def.	+				+		+	Poor growth, hyponatremia/hyperkalemia, variable phenotype
Crigler-Najjar syndrome type 1	+						+	Kernicterus w/t neonatal diagnosis and phototherapy. Lethal w/t liver transplant
Galactosemia, Duarte and Duarte/classical variants	+		+			+	+	Carriers of classical gene identified by NeoGen. Common in Maryland Amish
Glutaryl CoA dehydrogenase deficiency	+		+		+	+	+	Basal ganglial degeneration with cerebral palsy-like dystonia
GM1 gangliosidosis, beta-galactosidase deficiency	+	+						Lethal. Found in Milroy Amish deme but not in Lancaster County
Medium chain acyl-CoA dehydrogenase deficiency	+		+		+	+	+	Rare. High morbidity and mortality w/t neonatal screenings. Infection induced crisis
3-Methylcrotonyl-CoA carboxylase deficiency	+		+		+	+	+	Mild phenotype, several asymptomatic adults and children
Phenylalanine hydroxylase deficiency	+		+	+	+	+	+	Variable phenotype, severe to mild hyperphe
Propionyl-CoA carboxylase deficiency	+		+		+	+	+	Variable Phenotype. Basal ganglial injury, cardiomyopathy
Pyruvate kinase deficiency	+	+					+	Common in Belleville deme. Erythroblastosis, hepatopathy and hemolysis
Short chain acyl dehydrogenase deficiency	+		+		+	+	+	Infants asymptomatic. Increased butyrylcarnitine and urine ethylmalonate
Sitosterolemia	+				+		+	Coronary artery disease in childhood. High cholesterol and sitosterol
Syndromes								
Arterial venous malformation syndrome	+							Common in Lancaster Amish. Intracerebral hemorrhages in all age groups. Dx by MRA
Blank syndrome, familial hypercholanemia	+						+	Poor growth, pruritis, vitamin K, and D deficiencies in infancy
Byler disease, familial intrahepatic cholestasis	+						+	Poor growth, pruritis, vitamin K, and D deficiencies in infancy. Lethal w/t liver transplant

Cartilage-hair hypoplasia dwarfism	+	+	+	Variable phenotype for growth, hair, immune deficiency, and Hirschsprungs disease
Cerebellar ataxia with preserved reflexes	+	+	+	Gross motor and language delays. Ataxia and abnormal gait variable
Cockayne syndrome	+	+	+	Lethal. Typical phenotype, infantile form. Seen in Amish in Belleville area
Lethal microcephaly with 2-ketoglutaric aciduria	+	+	+	Common in Lancaster. Lethal with brain malformations and progressive encephalopathy
Congenital supraventricular arrhythmias	+	+	+	Severe arrhythmias. Life-threatening cardiac events in the neonate. Dx by EKG
Ectrodactyly, omphalocele and cardiac malformations	+	+	+	Rare, lethal syndrome. Some infants have ectodactyly only
Ellis-van Creveld dwarfism	+	+	+	Common in Lancaster. Pulmonary hypoplasia, cardiac disease in 25–40%
Fuchs endothelial dystrophy of the cornea	+	+	+	Presents in young adults as early glaucoma and cataracts
Mckusick-Kauffman syndrome	+	+	+	Female infertility without early detection and repair
Moyamoya disease	+	+	+	One case in Belleville Amish. The child also had pyruvate kinase deficiency
Muscular dystrophy, limb-girdle type			+	Beta-sarcoglycan glycoprotein defect
Neurofibromatosis			+	Uncommon. Two cases with: periobital and facial tumors; obstructive hydrocephalus
Osteogenesis imperfecta with opalescent teeth, fractures	+	+	+	Common. Dominant variant. Fractures later onset and osteoporosis
Polycystic kidney disease, adult type			+	Onset renal failure age 30–50 years. No liver disease. Renal transplant
Rett syndrome	+	+	+	Typical progressive microcephaly in females. Sporadic, full disability
Riehl syndrome, Pelizaeus-Merzbacher-like dysmyelination	+	+	+	Autosomal recessive variant. Congenital nystagmus, motor delays. Dx by MRI
Swarey syndrome, fetal gonadal failure, sudden infant death	+	+	+	Common in Belleville area. Lethal. Not endocrinopathy or Kennedy syndrome
Troponin type 1 myopathy, infantile nemalin myopathy	+	+	+	Common, lethal. Tremors in utero and at birth. “Chicken breast disease”

^aGene and mutation identification Puffenberger, 2003.

TABLE III. Genetic Disorder of Pennsylvania Memmonites

	Illness during infancy	History and exam	Neo gen or PA screen	Serum or (urine) amino acid analysis	Urine or (serum)		Diagnosed by mutation detection ^a	Clinical notes
					organic acids by GC/MS	Amino acids and acyl carnitines by MS/MS		
Metabolic disorders								
Crigler-Najjar syndrome type 1	+	+					+	Kernicterus w/t neonatal diagnosis and phototherapy. Lethal w/t liver transplant
Cystinuria	+			(+)			+	Renal stones in early childhood with risk of renal damage. Common. Four mutations
Gilbert syndrome							+	Common in families with Crigler-Najjar disease. Homozygotes for TATA box polymorphisms
Glycogen storage disease, type 6							+	Abnormal liver enzymes and high cholesterol. Irritability and hepatomegaly after weaning
Immune deficiency, SCID variant	+	+					+	ALC < 100/mm, no thymic shadow or T-lymph. Bone marrow transplant. ?IL7 receptor defect
Branched chain 2-keto acid dehydrogenase deficiency	+		+	+	+	+	+	Classical MSD. Liver transplant was effective therapy, bone marrow transplant was not
Medium chain acyl-CoA dehydrogenase deficiency	+		+	+	+	+	+	High morbidity and mortality without neonatal screenings. Infection induced crisis
3-methylcrotonyl-CoA carboxylase deficiency	+		+	+	+	+	+	Mild phenotype, several asymptomatic adults and children
Mevalonate kinase deficiency	+				+	+	+	Neonatal jaundice, erythroblastosis. Compound heterozygote
Phenylalanine hydroxylase deficiency	+			+	+	+	+	Variable phenotype, mild hyperphre to severe
Propionyl-CoA carboxylase deficiency	+		+	+	+	+	+	Same mutation as Amish. Variable Phenotype
Tyrosinemia type 3			+	+	+	+	+	One case, no clinical disease
Syndromes								
Dystonias DYT-1		+					+	Childhood onset, Israeli mutation, one case. DYT6 reported in midwestern Menn. group
Familial periodic fever (TRAPS)	+	+					+/-	Tumor necrosis factor receptor mutation in 1 family. TRAPS and FMF genes nl in another family
Fragile x syndrome		+					+	Common disorder. Variable phenotype makes clinical diagnosis difficult
Hereditary spherocytosis	+						+	Hemolysis, hyperbilirubinemia, abnormal blood smear. RBC fragility test diagnostic
Hirschsprung disease	+	+					+/-	Neonatal bowel obstruction and sepsis. Polygenic disease. Highly variable phenotype
Nephrotic syndrome, congenital	+						+	Edema and infections. Lethal w/t renal transplant
Osteogenesis imperfecta, detached retinae	+	+					+	Congenital detachment of retinae. Fractures, late onset. Unknown mutation

Osteogenesis imperfecta, Amish variant	+	+	Fractures, later onset
Rett syndrome	+	+	Typical progressive microcephaly in females Sporadic, full disability
Retinitis pigmentosa, areflexia, sensory ataxia	+	+	Common syndrome. AXPC1, Mapped to 1q31-32. No mutation in Plexin A2. Unknown mutation
Werdnig-Hoffman disease, infantile	+	+	Common, lethal SMN1 exon 7 deletion. A milder clinical variant has been seen in one family
Familial manic-depressive illness	+	+	Variable phenotype, common. Childhood onset does occur. Mutation unknown

^aGene and mutation identification Puffenberger, 2003.

allow the communities to care for their own. This desire was in response to the

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many difficulties encountered by the Plain people in getting access to, and paying for, specialized medical care. The origins of community support and the economic and medical impacts of the clinic can be understood in relation to the history of MSUD in the Mennonite people. The collection of essays and letters *What Is Wrong with Our Baby?*, compiled and published by John and Verna Martin, parents of two MSUD children, is an interesting and sometimes sobering look at modern health care from a parent's perspective [Martin and Martin, 1995]. The oldest surviving Mennonite patient with MSUD was born in 1967, but many undiagnosed or untreated infants died before she was born. She was in a hospital in Philadelphia for 6 months, did not regain her birth weight for 4 months, and today has severe disability and retardation. Her sister, in whom MSUD was recognized by 3 days of age, became severely intoxicated, remained hospitalized for many weeks, and also has profound retardation.

FINANCIAL DIFFICULTIES FOR PLAIN PEOPLE IN TRADITIONAL MEDICAL SETTINGS

Between 1966 and 1988, 36 Mennonite children were born with MSUD. Over these 22 years, the management of the neonates improved and hospitalization times shortened. However, as funding for research in biochemical causes of mental retarda-

tion waned, the direct costs of care paid by the Mennonite community increased sharply. By 1988, the initial care for a newly diagnosed neonate with MSUD in regional pediatric hospitals was \$40,000–\$60,000 or more [Morton et al., 2002b]. Amino acid analyses cost \$450, and when coupled with physician charges and other testing charges, the total cost of an outpatient evaluation was typically \$1,000–\$1,500. These expenses were a major difficulty for the Plain people, as they historically have not participated in medical insurance programs. Approximately half of the families contribute to a form of major medical insurance called Amish Aid or Mennonite Aid, which is administered by businessmen within the churches. Prior to the opening of the clinic, benefit auctions were held to help pay hospital bills of \$50,000 or higher. Other church districts collect alms to help families with large medical bills. Members of the Old Orders are not eligible for Medicare or Medicaid benefits because of an exemption from Social Security taxes. When young adults join the Old Order Amish or Mennonite churches, they sign an *IRS Form 4029*, which indicates that they are “a member of a religious body that is conscientiously opposed to social security benefits but that makes reasonable provision for its own dependent members” [Hostetler, 1993]. The exemption from the tax also means that those who sign *IRS Form 4029* agree not to accept money from state or federal programs that are paid for by Social Security funds. This cultural practice is one of many issues community members face when obtaining care for their children.

CHALLENGES IN THE MEDICAL MANAGEMENT OF THESE DISORDERS

Earlier diagnosis and improved management did, in many cases, allow normal growth and development, yet several otherwise healthy children with MSUD died unexpectedly from acute cerebral edema after the newborn period. Of the 36 children with MSUD

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born between 1966 and 1988, 14 (36%) died of cerebral edema before the age of 10 years. The typical course of the children was that they became ill with a common infection, had poor appetite, vomiting, and irritability, which progressed to lethargy, and then to coma. By the time arrangements were made to travel from Lancaster to the regional hospital, the biochemical intoxication was severe. Emergency efforts to reverse the intoxication by intravenous fluids, glucose, and dialysis failed, the cerebral edema worsened, and within 24 hr after entering the hospital, the child died of brain stem herniation. Physicians and parents knew that common infectious illnesses provoked serious metabolic intoxication, but little was known about how to control the metabolic response to such illnesses in the outpatient setting. Local services for children were limited. Specialists and equipment that were needed to study the early stages of cerebral edema were in research laboratories far from the patients. The available biochemical testing was of no utility for the rapid treatment necessary to avert cerebral edema because results took as long as 2 weeks. Routine outpatient visits to metabolic clinics in Philadelphia could only be made on one afternoon each week, and outpatient services to assess acutely ill children were not available. Instead, ill children were referred to emergency rooms, and the medical staff of the ER usually knew less about the management of MSUD than did the child's parents. Newborn screening for MSUD was started by Pennsylvania in 1988, but the state-administered pro-

gram did not substantially improve matters for these patients. Until the Guthrie method was replaced by the tandem mass spectrometry program in 1993, false positive rates were unacceptably high [Morton et al., 2002b]. The state program made no provisions to test and provide immediate results for high-risk infants. The three Mennonite infants who were found by the state program were 5 and 6 days old and already ill. More important, the state follow-up program made no provisions to pay for the care of ill neonates, had no plans for the assessment and care of acutely ill older children, did not address the problem of cerebral edema, and did not support care for patients over the age of 21 years. The state program did offer to pay for several amino acid levels per year and offered formula to those who qualified for, and would accept, Medicaid, but the program required that Mennonite families obtain these services at the hospitals in distant regional centers.

In 1990, an amino acid analyzer was given to the Clinic for Special Children by two Mennonite churches. To date, the clinic has performed 16,402 amino acid analyses on this instrument. Because of our nonprofit status and community support, we were able to reduce the patient test charge from \$450 to \$50. We also decreased the analysis and reporting time for plasma branched-chain amino acids to 20 min, and we routinely quantify plasma amino acids during an outpatient clinic visit. Between 1989 and 2003, we managed 42 neonates with MSUD. Twenty-one of these neonates were from high-risk couples recognized by family histories or carrier tests, and the infants were tested for MSUD and diagnosed before 24 hr of age. With timely diagnosis, the neonates were never ill and could be managed without hospitalization. The diagnosis of MSUD was made in 20 additional neonates diagnosed because of clinical illnesses; 3 of these infants were found by the Pennsylvania screening program. These 20 ill infants were managed at Lancaster General Hospital by the clinic staff and had a typical stay of 3–

5 days with a hospital cost of \$1,000–2,000 per patient per day. Over a 12-year period, developmental outcomes have been good [Morton et al., 2002]. We currently follow more than 70 patients with MSUD, most of whom are of Mennonite descent. Between 1988 and 2002 we had 137 admissions to our hospital for acute MSUD intoxication; however, the average annual rate of hospitalization for our MSUD patients is less than 1 day per patient-year of follow-up. Between 1988 and 2002, there were, however, no deaths from cerebral edema. The current total annual formula cost for our MSUD patients is about \$115,000. Again, most Mennonite families do not accept formula or reimbursement for costs from the Pennsylvania MSUD follow-up program. The clinic pays half of these costs for families who have limited resources, and the families pay the balance. The charge for an outpatient visit is \$35, and medical care and laboratory testing are provided at the clinic regardless of a family's ability to pay. In summary, a comprehensive health care system was designed to provide sophisticated medical care of patients with MSUD within their community. By combining community support and other donations with knowledgeable and sophisticated laboratory and clinical care, the morbidity, mortality, and costs have been reduced.

CARE FOR PATIENTS WITH DISORDERS OTHER THAN MSUD

We believe that the cost of operating the Clinic for Special Children could be fully justified if we cared for no other patients than Mennonite children with MSUD. However, the medical and economic impacts of care for patients with GA1, MCADD, Crigler-Najjar disease, the bile salt disorders, and many other disorders that affect Amish and Mennonite children provide equally compelling justification for the clinic. More than 95% of the disorders we see cause clinical problems during infancy. Ten of the syndromes

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can be recognized by history and physical examination, including the common disorders, Amish lethal microcephaly [Kelley et al., 2002; Strauss et al., 2002], Swarey syndrome [Morton et al., unpublished results], a variant of cerebellar ataxia [Morton et al., unpublished results], and a newly described syndrome with infantile retinitis pigmentosa and sensory ataxia [Higgins et al., 1999]. Five disorders are primarily diagnosed by amino acid analysis, and eight disorders by organic acid analysis with gas chromatography/mass spectrometry (GC/MS). Genomic DNA mutation analysis is performed in the clinic [Puffenberger and Morton, 2002; Puffenberger, 2003] and is now used to diagnose 19 disorders, including Byler disease and two other disorders that disrupt bile salt transport, Crigler-Najjar disease, fragile-X syndrome, glycogen storage disease type VI [Chang et al., 1998], Amish osteogenesis imperfecta, infantile spinal muscular atrophy, and methylenetetrahydrofolate reductase deficiency [Puffenberger and Morton, 2002]. Two common lethal disorders, troponin T1 myopathy [Johnston et al., 2000] and infantile spinal muscular atrophy, can be difficult to diagnose in a newborn by examination, and we now routinely confirm these diagnoses by mutation analyses. Neonatal metabolic screenings done by Neo Gen Inc. identify using tandem mass spectrometry routinely identify 14 of the disorders that we see. The preliminary biochemical diagnoses for most of these disorders is confirmed in our laboratory on the original specimen through polymerase chain reaction (PCR)-based mutation detection. The molecular basis of 17 disorders seen at the clinic remains to be identified. Seven of these disorders should yield to candidate gene sequencing, including GM1 gangliosidosis, Bartter disease, and polycystic kidney disease, whereas 10 syndromes without candidate genes will require genome-

wide studies, including the cerebral arteriovenous malformation syndrome, manic depressive illness, several congenital deafness syndromes, and a common seizure/mental retardation syndrome.

THE IMPORTANCE OF PRESYMPTOMATIC CARE

In our clinic, we have emphasized the importance of diagnosis of asymptomatic infants. For example, the diagnosis of GA1 after degeneration of the basal ganglia allows no opportunity for effective treatment [Morton et al., 1991; Strauss and Morton, 2003a; Strauss et al., 2003b]. The prospective management of GA1 prevents basal ganglial degeneration in at least 75% of cases. The clinical importance of recognizing asymptomatic infants with MCADD, propionic acidemia, Crigler-Najjar disease, adrenal insufficiencies, Bartter disease, the bile salt transport disorders, and galactosemia is readily apparent to a pediatrician who has cared for infants with these disorders who presented undiagnosed and severely ill.

We have also found it helpful to be able to immediately diagnose lethal disorders in neonates. GM1 gangliosidosis can be diagnosed by physical exam and a single inexpensive test of urine for increased urine oligosaccharide excretion. Infantile spinal muscular atrophy and troponin T1 deficiency are both routinely diagnosed in neonates by molecular methods at a charge of less than \$50 [Puffenberger and Morton, 2002]. Because of easy access to the clinic, prolonged hospitalizations involving multiple invasive and expensive diagnostic tests for lethal disorders can usually be avoided.

In the primary care setting, undiagnosed genetic syndromes present differently than in genetic clinics at referral centers. Most often, the infants who are seen at the clinic are irritable, feed poorly, gain weight slowly, and in other less well defined ways seem to the parents to be "just not right." Many teenage patients and adults in these populations with underlying genetic syndromes had not been evaluated by modern diagnostic methods. The first Mennonite girl

found to have MCADD at the clinic was 14 years old and had been hospitalized several times because of vomiting and ketotic hypoglycemia. Her sibling died at age 6 months with hypoglycemia and a fatty liver, which was diagnosed as Reye syndrome in 1976.

Here and elsewhere, some genetic disorders have been misdiagnosed as child abuse. Approximately 10% of patients with GA1 whom we have seen were originally thought to be victims of child abuse. Infants with GA1 may present with subdural hemorrhages after minor head trauma, because 75% of the infants have open subdural and subarachnoid spaces that are crossed by bridging veins [Strauss and Morton, 2003a]. These intracranial hemorrhages are usually large, and retinal hemorrhages have developed spontaneously in association with large intracranial hemorrhages [Hymel et al., 2002]. In the Amish population of Lancaster County, we follow 28 children with three different hepatic bile salt transport disorders. Nine of these infants (30%) presented before 6 months of age with bruising and bleeding secondary to vitamin K deficiency. One such infant presented in a coma with a full fontanel, retinal hemorrhages, and bruising on bony prominences. Shaken baby syndrome was the suspected diagnosis. The infant was subsequently found to have a vitamin K-responsive coagulopathy, high serum bile salts, a deep, intracerebral hemorrhage, and unilateral retinal hemorrhages, which were atypical of shaken baby syndrome. This infant and her sibling are now known to be homozygotes for a mutation in the tight junction protein 2 gene [Carlton et al., 2003]. Fractures caused by relatively mild types of osteogenesis imperfecta found in the Plain populations have also been mistaken for child abuse.

THE UTILITY OF A DETAILED UNDERSTANDING OF THE NATURAL HISTORY OF DISEASE

We have had a unique opportunity to observe the natural history of these

disorders in many patients. The Menonite variant of MSUD arises from a stop codon in exon 1 of the *BCKDHA* gene. Thus far, all Menonite patients have been homozygous for this mutation and all have the classical form of the disorder. In spite of the mutational homogeneity, the phenotype ranges from patients who are profoundly disabled to those who are apparently normal. Poor outcomes in patients with MSUD can usually be understood in terms of one or more of the following problems: delays in neonatal diagnosis and treatment, chronic essential amino acid deficiencies, ineffective management of metabolic intoxication during infectious illnesses, and acute vascular brain injuries caused by cerebral edema. Neonates with MSUD who are diagnosed before 24–36 hr of age are not ill, and progressive metabolic intoxication can be prevented by rational management [Morton et al., 2002b]. Plasma amino acid quantification allows diagnosis of the disorder as early as 12 hr of age if high-performance liquid chromatography (HPLC) or tandem mass spectrometry is used. It is important to note that the Guthrie bacterial inhibition assay is not sufficiently sensitive to be used for specimens collected before 24 hr of age [Morton et al., 2002b]. All infants with classical MSUD identified by newborn screening programs will be very ill if screening results are not available before 4 days of age. Infants identified and treated between 4 and 10 days of age can have good neurological outcomes, but the cost of initial treatment is higher than those diagnosed within 24 hr. Neonatal screening programs for MSUD wherein specimens are not collected until 72 hr of age and results are not reported before 14 days of age can expect high initial costs of treatment and high morbidity and mortality. After MSUD is diagnosed, the prognosis for the patients depends on several variables, including the time required to lower the plasma and tissue leucine levels to normal, prevention of the vascular compression and ischemic injuries caused by critical brain edema, and prevention of prolonged or severe brain and systemic essential amino acid

deficiencies. Our impression is that prolonged deficiencies of valine in the brain during the critical period of rapid brain growth between birth and 2 years of age may be a major factor in mental retardation seen in children with poorly treated MSUD [Morton et al., 2002b].

The mainstay of therapy for MSUD has been the dietary restriction of the branched-chain amino acids. Unfortunately, in many follow-up programs, leucine levels are measured infrequently and isoleucine, valine, and the other essential amino acids are not monitored at all. It is not possible to understand the relationship of day-to-day metabolic control and neurological outcome when only a few plasma leucine levels are measured per year. Intermittent changes in tolerance related to normal variation in growth velocity or illnesses will not be appreciated. Deficiencies of branched-chain amino acids and the unbalanced transport of the other neutral amino acids that compete with leucine for entry into the brain cannot be assessed by isolated measurements of leucine. The effects of dietary therapy on intellectual outcome can only be understood by reference to the cumulative effects of amino acid metabolism throughout the critical period of brain growth and development. In recent years, we have monitored blood amino acid levels in rapidly growing infants twice weekly and gained an appreciation of how quickly amino acid concentrations change, especially during otherwise minor infectious illnesses.

Over a 13-year period, we have admitted patients with MSUD to the hospital for management of metabolic crisis 137 times. More than 120 of these admissions were necessary because of catabolic intoxication caused by infectious illnesses such as gastroenteritis, otitis media, streptococcal pharyngitis, sinusitis, and pulmonary infections. Appendicitis, cholecystitis, torsion and infarction of the ovary, and fractures also cause metabolic crises. In addition, we have 100–200 MSUD outpatient visits per year to manage infectious illnesses and other stressors. These illnesses cause significant metabolic changes in patients with MSUD and require one or more

measurements of plasma amino acids and changes to a different treatment regimen to prevent prolonged or progressive metabolic intoxication. The ultimate impact of these illnesses on clinical outcome is influenced by the rapid availability of outpatient and inpatient services, including amino acid analysis, MSUD hyperalimentation solution, and medical care by physicians and nurses who have experience with the management of the metabolic crisis and cerebral edema.

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MEDICAL CARE AND THE OUTCOME OF OTHER GENETIC DISORDERS

Eleven of the disorders that affect the Amish and Menonites are lethal or invariably cause full disability. Although these 11 disorders are lethal, affected children may live months or years and require many human and medical services. The clinic routinely provides medical care for children who have lethal diseases. Most of these children are cared for, and die, at home. Much of the community support for the clinic comes from families who need and appreciate this care. The other 51 metabolic disorders and syndromes present with a range of clinical problems that require timely diagnosis and ongoing medical care. Our daily practice includes management of episodic crises

in patients with the adrenal insufficiency syndromes, Crigler-Najjar syndrome, and various organic acidemias; and the chronic malnutrition and fat-soluble vitamin deficiencies of the bile salt disorders, as well as the cardiac and pulmonary problems of the patients with Ellis-van Creveld syndrome and the immune and gastrointestinal disorders in patients with cartilage-hair hypoplasia. The early diagnosis of genetic syndromes and anticipation of predictable problems is medically important and often limits the adverse effects of the disorder on the life of the patient, especially for metabolic disorders, but also for many other nonmetabolic syndromes.

Immune deficiency states are known to be associated with several of the disorders that affect the Plain people. In infants with cartilage-hair hypoplasia, we have encountered severe varicella infections, *Pneumocystis carinii* pneumonia, *Hemophilus influenzae* meningitis in an immunized 4-month-old, and persistent parvovirus infection with a chronic inflammatory disease similar to juvenile rheumatoid arthritis. Our young patients with Ellis-van Creveld dwarfism who have cardiac lesions and pulmonary hypoplasia are particularly vulnerable to infections with respiratory syncytial virus. Our first encounter with the Amish-Mennonite variant of propionic acidemia was an undiagnosed 12-month-old who presented with a viral respiratory tract infection and developed acute striatal necrosis and a dilated cardiomyopathy with endocardial fibroelastosis. Infants with the Mennonite variant of nephrotic syndrome [Bolk et al., 1999] typically die from the immune deficiency state caused by the loss of immune globulins and complement rather than from the loss of albumin or renal failure. Gram-negative sepsis must be anticipated in the ill neonate with galactosemia or Hirschsprung disease. Based on our experience in Lancaster County, the practice of medical genetics cannot be separated from general pediatrics, and much of our work in general pediatrics is focused on the prevention and treatment of infectious illnesses. The disease course of

many genetic disorders is directly determined by how effectively the interaction of infectious disease and underlying genetic disorder is understood and managed.

BIOLOGY OF GENETIC DISORDERS

Our daily clinical work within a population with high incidences of genetic diseases provides a unique opportunity to study the biology of genetic disorders. Because of the allelic homogeneity of many disorders among the Plain people, we observe a consistent, or at least much more limited, spectrum of variation at the primary disease-causing locus than do most physicians. These observations have given us a different perspective on genotype-phenotype correlation. MSUD exemplifies the complex relationship of phenotype and genotype and, in addition, the manner in which health care delivery affects clinical outcome. The description of gene mutations is useful for the diagnosis of many disorders, but mutation analysis alone has seldom provided us with insight into the phenotypic diversity that we encounter in our patients who share the same disease genotypes. Our understanding of variations in the natural history of genetic disorders is more often based on the concepts and language of cell biology, pathophysiology, and critical periods of brain development than on specific molecular lesions. MSUD provides a useful example of the complex relationship between gene and disease. Descriptions of mutations in branched-chain keto-acid dehydrogenase as an underlying cause of MSUD do not help us understand the multisystem regulators of protein anabolism or catabolism that cause marked changes in leucine tolerance. These perturbations of anabolism and catabolism are more important influences on day-to-day metabolic control and illness than are the underlying mutations. Nor do the mutations explain leucine-induced encephalopathy, acute cerebral edema, or mental retardation. The biology of MSUD can only be understood in terms of the acute

and chronic effects of high blood and tissue concentrations of leucine on protein turnover in muscle and liver, cell volume regulation, and brain amino acid homeostasis as it relates to neurotransmitter function, and the development and maintenance of brain structure. MSUD arises from a complex derangement of interorgan amino acid transport.

Our impression is that scientists who work as physicians and care for several patients with the same genetic disorder over long periods of time will develop a different understanding of genetic disease than scientists who study disease mechanisms only in the laboratory. Laboratory scientists implicitly assume that a genetic disease can be adequately characterized by molecular, enzymatic, biochemical, and cellular experiments that are done at one point in time and are independent of experience with an individual patient. Our experience suggests that descriptions of disease mechanisms based on in vitro studies are necessarily incomplete and may lead to an oversimplification of models of genetic disorders. MSUD, GA1, and the majority of the other disorders managed at the clinic can only be understood in terms of the pathophysiological mechanisms that unfold in individuals over significant periods of time and in relationship to many different disease-causing factors, many of which are not genetic in origin. For GA1, MSUD, and PKU, the neurological syndromes caused by single-gene mutations arise from mechanisms of injury and dysfunction that are linked to critical periods of brain development. In patients with GA1, episodes of metabolic illness that cause acute striatal necrosis in an infant are well tolerated in the 6-year-old or adult. Unbalanced amino transport that leads to mental retardation in the infant with PKU and MSUD causes attention deficit disorder, mood disorders in older patients, and presenile dementia in adults. The single-gene mutations of MSUD and PKU give rise to diseases by disruptions of complex biological processes such as the metabolic adaptations to fasts and illnesses, cell volume regulation, brain amino acid

homeostasis, and postnatal organ growth and development. Description of a disease process without reference to such biological processes and only by reference to point mutations in genes or changes in enzyme activities seems to us, as clinicians, to be inadequate. Assumptions about the nature of genetic disease and the terms that are used to characterize these disorders influence how physician-scientists are trained and how, throughout their careers, these scientists ask and answer questions. A recent editorial in the *Journal of Clinical Investigation* indicated that research support in the past 20 years has overwhelmingly directed physician-scientists to the laboratory and away from patients, showing that 97% of physician-scientists who received the Howard Hughes clinical research awardees elected to do research that did not involve patients [Goldstein and Brown, 1997]. Our experiences at the clinic over the past 14 years suggest many important aspects of genetic diseases will only become apparent to physician-scientists who care for individual patients over long periods and who are interested in patients, pathophysiology, and biology in the most general sense.

CONCLUSIONS

The Clinic for Special Children was established to provide care for Mennonite and Amish children in Lancaster County who have MSUD and GA1. However, the need for a similar approach to care for patients with other disorders, in other places and populations, is increasingly apparent. Our Mennonite patients with MSUD come from every region of Pennsylvania, Maryland, New York State, Kentucky, Michigan, Wisconsin, Indiana, and Iowa. GA1 is found in all Amish populations of Pennsylvania and in northern New York State. The most recent cases of GA1 we have seen were recognized through the supplemental screening program but were not of Amish descent. In the general population of the United States, GA1 is now known to be one of the more common inherited metabolic disorders [Strauss and Morton, 2003].

Around the world, clusters of cases of GA1 caused by founder genes have been identified in Ireland, Spain, Israel, Saudi Arabia, Chile, and two Native American populations. Regardless of the underlying mutation, diagnosis and treatment of MSUD and GA1 before the onset of cerebral edema are essential. The medical care of a patient with a genetic disorder is no different than the care of a patient with other problems that are medically complex, such as diabetes mellitus, manic depressive illness, leukemia, congenital heart disease, renal failure, rheumatoid arthritis, seizures, congenital and acquired immune deficiency syndromes, drug dependency, and many other chronic medical troubles. Rare genetic disorders are like the majority of diseases treated by physicians: few curable diseases exist. Nonetheless, accessible and effective medical care can limit suffering, improve function, reduce dependency, and otherwise limit the effect of disease, genetic and acquired, on the life of a patient. William Osler said, "Such is our work."

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