ORIGINAL ARTICLE

Transplantation of Umbilical-Cord Blood in Babies with Infantile Krabbe's Disease

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ABSTRACT

BACKGROUND

Infantile Krabbe's disease produces progressive neurologic deterioration and death in early childhood. We hypothesized that transplantation of umbilical-cord blood from unrelated donors before the development of symptoms would favorably alter the natural history of the disease among newborns in whom the disease was diagnosed because of a family history. We compared the outcomes among these newborns with the outcomes among infants who underwent transplantation after the development of symptoms and with the outcomes in an untreated cohort of affected children.

METHODS

Eleven asymptomatic newborns (age range, 12 to 44 days) and 14 symptomatic infants (age range, 142 to 352 days) with infantile Krabbe's disease underwent transplantation of umbilical-cord blood from unrelated donors after myeloablative chemotherapy. Engraftment, survival, and neurodevelopmental function were evaluated longitudinally for four months to six years.

RESULTS

The rates of donor-cell engraftment and survival were 100 percent and 100 percent, respectively, among the asymptomatic newborns (median follow-up, 3.0 years) and 100 percent and 43 percent, respectively, among the symptomatic infants (median follow-up, 3.4 years). Surviving patients showed durable engraftment of donor-derived hematopoietic cells with restoration of normal blood galactocerebrosidase levels. Infants who underwent transplantation before the development of symptoms showed progressive central myelination and continued gains in developmental skills, and most had age-appropriate cognitive function and receptive language skills, but a few had mild-to-moderate delays in expressive language and mild-to-severe delays in gross motor function. Children who underwent transplantation after the onset of symptoms had minimal neurologic improvement.

CONCLUSIONS

Transplantation of umbilical-cord blood from unrelated donors in newborns with infantile Krabbe's disease favorably altered the natural history of the disease. Transplantation in babies after symptoms had developed did not result in substantive neurologic improvement.

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KRABBE'S DISEASE, OR GLOBOID-CELL leukodystrophy, is an autosomal recessive disorder due to deficiency of the lysosomal enzyme galactocerebrosidase and characterized by failure of the process of myelination in the central and peripheral nervous systems, rapidly progressive neurologic deterioration, and death. More than 60 mutations have been identified that result in low enzymatic activity leading to a decreased ability to degrade galactolipids found in myelin. The accumulation of galactolipids results in inflammation, dysmyelination, and demyelination of the developing brain. In the infantile form, symptoms appear before six months of age and include irritability, dysphagia, progressive spasticity, mental deterioration, blindness, deafness, seizures, and death, usually before two years of age.[1]

Allogeneic hematopoietic stem-cell transplantation has been previously reported to be beneficial in patients with early stages of juvenile Krabbe's disease.[2] Donor stem cells repopulate various tissues, delivering enzymes both inside and outside the vascular compartment; children so treated have had improved neurologic outcomes and improved overall survival.[2-4] Bone marrow has traditionally been used as the source of donor stem cells for transplantation. However, many children lack a matched donor, and recruitment of an unrelated adult donor takes too long for the treatment of a rapidly progressive disorder. Banked umbilical-cord blood from unrelated donors is readily available and can be used after myeloablative therapy.[5-7]

We assessed the safety and efficacy of transplantation of umbilical-cord blood from unrelated donors with partial HLA mismatches for the treatment of two groups of infants with Krabbe's disease. Krabbe's disease was diagnosed prenatally or at birth because of a family history of the disease in 11 patients, and they underwent transplantation as newborns; 14 children without a family history of the disease underwent transplantation in infancy after the onset of clinical symptoms.

METHODS

PATIENTS

Between August 1998 and August 2004, 11 asymptomatic newborns and 14 symptomatic infants with Krabbe's disease underwent transplantation of umbilical-cord blood from unrelated donors. The disease was diagnosed in six newborns prenatally and in five shortly after birth. The disease was diagnosed in the 14 symptomatic patients when they were between four and nine months of age. Treatment plans were approved by the institutional review boards of Duke University Medical Center, Durham, North Carolina (22 patients), Cardinal Glennon Children's Hospital, St. Louis (1 patient), DeVos Children's Hospital, Grand Rapids, Michigan (1 patient), and Hôpital Sainte-Justine, Montreal (1 patient), and written informed consent was obtained from the parents of all infants. Four patients were enrolled in the Cord Blood Transplantation Study. Assays of leukocyte galactocerebrosidase activity confirmed the diagnosis in all patients.

SELECTION OF HLA-MATCHED UNITS

Searches for cord-blood units from unrelated donors were conducted through the National Marrow Donor Program, the Cord Blood Transplantation Study banks, and the New York Blood Center. Intermediate-resolution typing for HLA class I alleles (A and B) and high-resolution typing for HLA class II DRB1 alleles were used for matching. The unit of cord blood had to deliver at least 3×10^7 nucleated cells per kilogram of body weight (the count before cryopreservation was used).[8] Units matching for four to six of six HLA antigens were tested for galactocerebrosidase[9] in 21 of the 25 patients; after 2 to 4 units were tested per patient, units with higher activity were selected when available. The cryopreserved units were thawed, washed, and tested for blood-borne pathogens, hemoglobinopathies, hematopoietic progenitor-cell content, and sterility, as previously described.[8,10]

TRANSPLANTATION PROCEDURE

Patients were prepared for transplantation with busulfan and cyclophosphamide. They received cyclosporine and steroids as prophylaxis against graft-versus-host disease and supportive care, as described previously.[8,11] Twenty-three patients received horse antithymocyte globulin; one received rabbit antithymocyte globulin. Myeloid engraftment was defined as occurring on the first of three consecutive days on which the absolute neutrophil count was above 500 per cubic millimeter with donor cells. Platelet engraftment was defined by a platelet count of at least 50,000 per cubic milliliter for at least seven consecutive days.

NEURODEVELOPMENTAL ASSESSMENT

Standardized and validated neurobehavioral tools were used to assess all infants before transplan-

tation and all surviving infants after transplantation.[12-19] The results were compared with norms for typically developing children. Nineteen patients were assessed both at the Clinical Center for the Study of Development and Learning, University of North Carolina at Chapel Hill, and at Duke University; one patient was evaluated at DeVos Children's Hospital Neurobehavioral Center; and one at Hôpital Sainte-Justine. Age equivalents were used to permit comparisons across tests and to identify the development of new skills. Cognition, adaptive behavior, receptive language, expressive language, gross motor skills, and fine motor skills were assessed.

MAGNETIC RESONANCE IMAGING

A neuroradiologist who was blinded to the clinical status of the patients reviewed all magnetic resonance imaging (MRI) scans of the brain for abnormalities at baseline and progression of myelination three months to six years after transplantation. Myelination was indicated by the development of a hyperintense signal on T_1-weighted axial images and a hypointense signal on T_2-weighted axial images in age-appropriate regions. These included the posterior limb of the internal capsule, the genu and the splenium of the corpus callosum, the corona radiata, the centrum semiovale, and the subcortical white matter.

NEUROPHYSIOLOGICAL STUDIES

Electroencephalography, nerve-conduction studies, and tests of flash visual evoked potentials (visual evoked potentials on delivery of flash stimuli to the eyes) and brain-stem auditory evoked responses were performed before transplantation and at scheduled intervals and interpreted according to the guidelines established by the American Electroencephalographic Society.[20] Electroencephalograms (EEGs) were considered abnormal if there was focal or generalized slowing or if spikes or sharp waves were present. The flash visual evoked potential was considered normal if the P100 wave was present, and abnormal if it was missing. The brain-stem auditory evoked responses were considered abnormal either if the interpeak latency of waves I to V was prolonged or if any of the obligate wave forms (I, III, or V) was missing. Results of nerve-conduction studies were considered abnormal if they showed prolongation of the distal latency, low amplitude, no evoked response, or prolonged latency of the F wave. Study results were interpreted by expert physicians blinded to the clinical status of the patients.

STATISTICAL ANALYSIS

The probability of event-free survival (defined as survival with durable engraftment of donor cells) was calculated by Kaplan–Meier analysis. We compared survival among the 11 asymptomatic newborns who underwent transplantation with that among the 14 infants who underwent transplantation after the onset of symptoms and in an untreated control group.[21] Survival data for the untreated group (190 patients) were provided by the Hunter's Hope leukodystrophy registry. The cutoff date for data analysis was January 28, 2005.

RESULTS

CHARACTERISTICS OF THE PATIENTS AND DONORS

After myeloablative chemotherapy, 11 newborns (4 boys and 7 girls) ranging in age from 12 to 44 days, with a median weight of 4.0 kg, and 13 of 14 symptomatic infants (8 boys and 6 girls) ranging in age from 142 to 352 days, with a median weight of 7.2 kg, underwent transplantation with banked umbilical-cord blood from unrelated donors with partial HLA mismatches (Table 1). One symptomatic infant had no mismatches. The median age at the initiation of myeloablative chemotherapy in the newborns was 18.5 days and that at transplantation was 28 days. The newborns received a higher median number of nucleated cells in units selected for transplantation than the older infants (22.07×10^7 vs. 17.24×10^7 cells per kilogram, respectively). After thawing, the median numbers of CD34 cells infused were 3.72×10^5 and 2.92×10^5 cells per kilogram, respectively (Table 2).

ENGRAFTMENT AND GRAFT-VERSUS-HOST DISEASE

Neutrophil and platelet engraftment in asymptomatic and symptomatic infants occurred a median of 17 to 18 and 57 to 70 days, respectively, after transplantation (Table 2). As of the last follow-up evaluation (median follow-up, 1024 days after transplantation), 16 of 17 surviving patients continued to have complete donor chimerism, whereas the 1 newborn patient who did not receive antithymocyte globulin continued to have stable mixed donor–recipient chimerism.

All surviving patients continued to have normal peripheral-blood galactocerebrosidase activity. The

Table 1. Characteristics of the Babies and the Unrelated Donors.*

Patient No.	Age at Transplantation	Sex	Weight	HLA Matches	HLA Mismatch	Blood Type		Pretransplantation Cerebrospinal Fluid Protein	Unrelated-Donor Galactocerebrosidase
						Patient	Donor		
	days		*kg*	*no./total no.*	*patient/donor*			*mg/dl*	*nmol/hr/mg of protein*
Asymptomatic newborns									
1	44	M	4.1	4/6	A30/25,B44/53	O–	A+	90	NA
2	20	F	4.2	4/6	A23/24,B50/07	O+	O+	NA	NA
3	37	F	4.8	4/6	A2/33,B39/bl	AB+	B+	299	3.2
4	22	F	3.8	5/6	B56/57	O+	O+	422	NA
5	29	M	3.9	4/6	B57/8,DRB14/13	B+	B+	183	4.5
6	36	F	4.1	5/6	B40/58	O+	A–	278	4.9
7	19	F	3.4	5/6	A26/2	A+	A+	NA	NA
8	28	M	4.2	5/6	B18/44	A–	A+	298	3.5
9	16	F	4.0	4/6	Abl/11,B39/18	O+	O+	89	3.4
10	13	M	2.8	5/6	B44/14	A–	A+	191	2.0
11	12	F	3.8	5/6	DRB19/8	O+	O+	239	2.3
Median			4.0						
Mean ±SD			3.9±0.5						
Symptomatic infants									
1	270	F	7.6	4/6	A32/23,B7/44	O+	O+	227	1.74
2	210	M	7.1	4/6	B62/45,BRB1 1103/1102	A+	A+	294	0.9
3	277	F	7.5	4/6	A24/2,B7/35	O–	O+	152	2.25
4	330	M	9.1	4/6	Abl/74,B39/63	B+	A+	176	2.29
5	210	M	5.8	4/6	A3/1,A11/31	A+	A+	891	NA
6	284	F	6.8	4/6	A3/22,B51/41	A+	O+	NA	1.4
7	173	F	7.0	4/6	A68/2,B40/51	A+	O+	259	2.7
8	207	M	7.6	5/6	DRB18/14	A+	O+	NA	NA
9	142	M	6.0	4/6	B44/57,DRB1 0401/0402	O+	O+	571	3.4
10	206	F	5.9	6/6	No mismatch	O–	A+	240	1.2
11	261	M	6.8	5/6	A3/30	A+	B+	187	3.9
12	153	M	7.2	5/6	A2/A24	B–	O+	222	4.8
13	282	F	8.5	5/6	DRB13/15	A–	O+	102	1.9
14	352	M	9.8	4/6	B14/35, DRB14/13	A–	B–	169	4.0
Median			7.2						
Mean ±SD			7.3±1.2						

* Units with matches for four to six of six HLA antigens were tested for galactocerebrosidase (normal range, 1 to 6 nmol per hour per milligram). Normal cerebrospinal fluid protein levels range from 70 to 120 mg per deciliter for newborns, and from 5 to 40 mg per deciliter for infants. NA indicates that no data were available, and bl denotes blank.

cerebrospinal fluid protein level was elevated in 7 of the 9 newborns and in all 12 symptomatic infants who were evaluated at the time of transplantation; it decreased gradually after transplantation but did not normalize in any patient (Tables 1 and 2). Grade I acute graft-versus-host disease (GVHD) developed in seven newborns, and grade II GVHD in one newborn. Moderate-to-severe acute GVHD

Table 2. Graft Characteristics and Outcomes after Transplantation of Umbilical-Cord Blood.*

Patient No.	No. of Cells		No. of CD34 Cells Infused	Grade of Acute GVHD	Site of Chronic GVHD	Time to Neutrophil Engraftment	Time to Platelet Engraftment	Time to Red-Cell Transplantation Independence	Post-Transplantation Cerebrospinal Fluid Protein	Post-Transplantation Galactocerebrosidase
	Cryopreserved	Infused								
	$\times 10^{-7}$/kg		$\times 10^{-5}$/kg			days			mg/dl	nmol/hr/mg of protein
Asymptomatic newborns										
1	21.93	14.20	1.80	I		14	53	49	104	2
2	37.81	26.90	8.60	II	Skin, AHA	10	61	64	NA	1.42
3	19.79	11.50	1.27	I		13	49	39	99	3.2
4	NA	17.00	2.18	0		29	31	31	200	3.38
5	39.20	25.00	5.65	I		20	101	115	157	3.5
6	17.36	15.84	3.72	I	Skin	20	67	53	86	5.6
7	65.57	25.76	13.00	0		17	47	33	NA	2.0
8	17.76	15.50	2.62	I		19	74	69	162	2.9
9	13.45	13.20	2.24	I	AHA	14	48	55	54	5.9
10	50.36	32.40	11.02	0		16	76	75	157	NA
11	22.20	13.20	14.51	I	AHA	25	64	62	NA	2.3
Median	22.07	15.84	3.72			17.0	57.0	54.0		
Mean ±SD	30.54±17.11	19.14±7.04	6.06±4.88			17.9±5.52	59.5±19.0	57.0±24.1		
Symptomatic infants										
1	22.76	16.40	10.99	I		12	45	35	204	1.2
2	14.40	8.87	2.84			31	NE	NE	149	1.42
3	16.88	7.73	1.31	IV		14	67	84	273	3.1
4	9.45	5.16	0.62			34	NE	NE	163	—
5	7.12	4.52	1.22	II	Skin, liver, AHA	22	61	50	135	0.65
6	9.52	7.96	1.31			NE	NE	NE	1113	—
7	17.6	12.97	4.32	NA		28	107	100	209	3.6
8	16.84	11.54	3.00	IV		18	NE	NE	199	—
9	24.33	19.50	11.70	I		15	58	60	204	3.2
10	16.07	11.46	3.94	I		15	56	63	143	0.8
11	19.60	13.77	2.48	II		29	106	52	449	2.0
12	26.45	17.35	2.76			30	NE	NE	126	0.8
13	21.72	16.50	5.26	II		15	36	48	254	5.9
14	23.09	22.30	7.36	I		14	90	91	155	4.1
Median	17.24	12.26	2.92			18.0	69.6	64.8		
Mean ±SD	17.56±5.9	12.57±5.39	4.22±3.5			21.3±8.0	61.0±26.0	60.0±22.0		

* Myeloid engraftment was defined by an absolute neutrophil count of at least 500 per cubic millimeter on three consecutive days. Platelet engraftment was defined by a platelet count of at least 50,000 per cubic millimeter without transfusion for at least seven consecutive days. Post-transplantation levels of cerebrospinal fluid protein decreased but remained abnormal. Galactocerebrosidase levels after transplantation were measured at scheduled intervals. Galactocerebrosidase levels were higher than the levels of the graft initially but at the last evaluation were similar to graft levels (range, 0.8 to 5.9 nmol per hour per milligram). The follow-up ranged from 4 to 66 months. AHA denotes autoimmune hemolytic anemia, NA no data available, and NE not evaluated. A dash indicates that the patient died before reaching the end point.

(grade II, III, or IV) developed in 5 of the 14 older patients. Limited, chronic GVHD of the skin developed in two newborns, and three had brief episodes of autoimmune hemolytic anemia that had resolved four months to two years after transplantation (Table 2).

SURVIVAL

As of January 28, 2005, all 11 newborns and 6 of 14 symptomatic infants had survived for a median of 36 and 41 months, respectively, after transplantation (Fig. 1). Survival among the newborns was better than among the untreated controls (P=0.001) or the symptomatic infants (P=0.01). Survival among the symptomatic infants was not statistically different from survival among the controls (P=0.28). Six of the newborns have outlived their affected siblings, and five have not yet reached the age at which the sibling died (Fig. 2). Complications after transplantation in the newborns included a catheter-related silent brain infarct diagnosed by MRI (in one patient) and asymptomatic or symptomatic hypertrophic cardiomyopathy (in two patients and one patient, respectively) that resolved as documented on serial echocardiograms after the discontinuation of steroids. In the symptomatic group, four infants died of progressive disease, one of GVHD, one of aspiration pneumonia, one of adenoviral infection, and one from complications after a liver biopsy for GVHD.

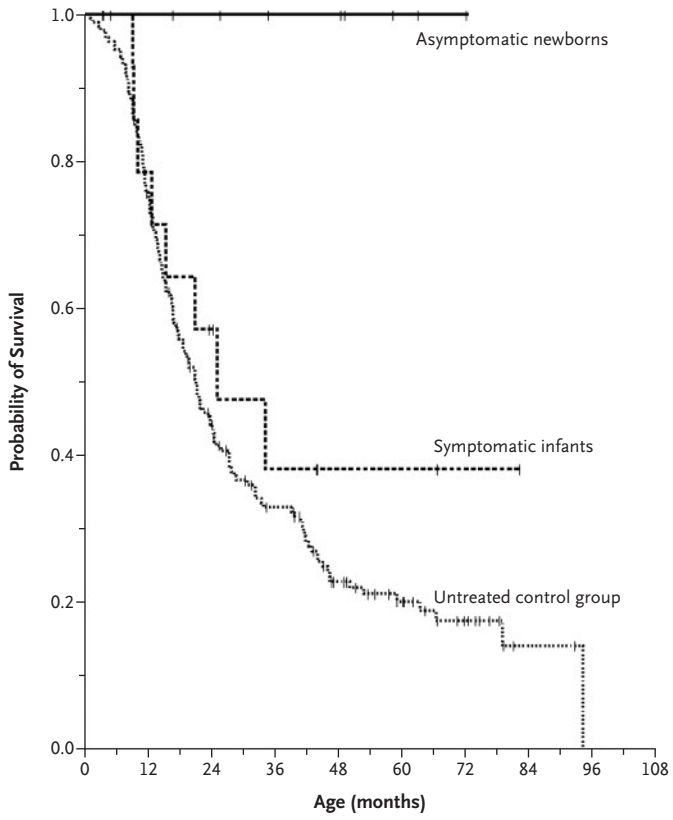

Figure 1. Kaplan–Meier Estimates of the Probability of Overall Survival among Patients with Krabbe's Disease.

Shown are Kaplan–Meier estimates of survival among all 11 asymptomatic newborns with Krabbe's disease who underwent transplantation of umbilical-cord blood from unrelated donors, as compared with 6 of 14 infants who underwent transplantation after the development of clinical symptoms (P=0.01) and 190 untreated affected babies (P=0.001). P=0.28 for the comparison between the symptomatic infants and the control group. The tick marks indicate the most recent follow-up for each patient.

NEUROLOGIC OUTCOMES

Brain MRI Scanning

The 11 children who underwent transplantation as newborns before the onset of symptoms each had two to seven follow-up scans during the period from six months to six years after transplantation. In all 11 children, brain MRI scans after transplantation showed normal progression of myelination, with age-appropriate changes in signal intensity in various white-matter sites. Pretransplantation MRI scans of three newborns showed abnormal hyperintense signals on T_2-weighted images in the posterior limb of the internal capsule; scans of another four newborns showed regions of abnormal hyperintense signal within the white matter adjacent to the lateral ventricles, consistent with dysmyelination. In these four babies, the regions of abnormal signal intensity decreased over time on serial scans (Fig. 3).

A total of 44 MRI scans from the symptomatic group (1 to 7 per patient) were available for review. On initial, pretransplantation MRI scans, obtained at four to seven months of age, the surviving symptomatic patients showed abnormal hyperintense signals on T_2-weighted images that were typically in the centrum semiovale, the corona radiata, and the white matter and dentate nuclei of the cerebellum. Thirteen of 14 patients had subsequent MRI scans three months to five years after transplantation, which showed disease progression in 12 patients, usually characterized by the development of brain atrophy, worsening hyperintense signal abnormalities on T_2-weighted images in the corona radiata, the centrum semiovale, and the posterior

limb of the internal capsule, and new signal abnormalities in the brain stem. The other patient's scan stabilized over time.

Visual Evoked Potentials

Studies of visual evoked potentials were available for eight newborns before and after transplantation. The results of three studies were initially abnormal but were normal by four months after transplantation. The remaining newborns had consistently normal visual acuity and function. Three patients were not studied because of the physician's preference.

Twelve of the symptomatic infants underwent testing of visual evoked potentials before and after transplantation. Eight of the 12 had abnormal results both times. Results of the studies of four patients were initially normal but became abnormal on follow-up. One patient underwent testing only before transplantation.

Brain-Stem Auditory Evoked Responses

Brain-stem auditory evoked responses were studied in 8 of 11 newborns before and after transplantation. The results were normal in four patients before transplantation and remained normal in two of the four patients after transplantation (follow-up range, 3 to 22 months). The responses were abnormal in the other four patients before transplantation and remained abnormal after transplantation (follow-up range, 3 to 16 months). All patients had normal hearing as measured by serial behavioral audiometry (visual-reinforcement audiometry and autoacoustic emissions) performed after transplantation.

Brain-stem auditory evoked responses were studied in 11 infants in the symptomatic group before transplantation, and all 11 had abnormal responses; 6 were retested one to five years after transplantation and still had abnormal responses.

Nerve-Conduction Studies

The results of nerve-conduction studies were abnormal in 9 of 11 newborns studied before transplantation. In seven of nine patients (studied four months to six years after transplantation), nerve conduction improved as compared with the pretransplantation results. Two other children showed initial improvement in the first 12 to 18 months after transplantation, but later studies showed that the results had worsened over time. In the symptomatic group, pretransplantation studies performed in 13 patients had abnormal results, and results were abnormal in 7 patients studied one to three years after transplantation.

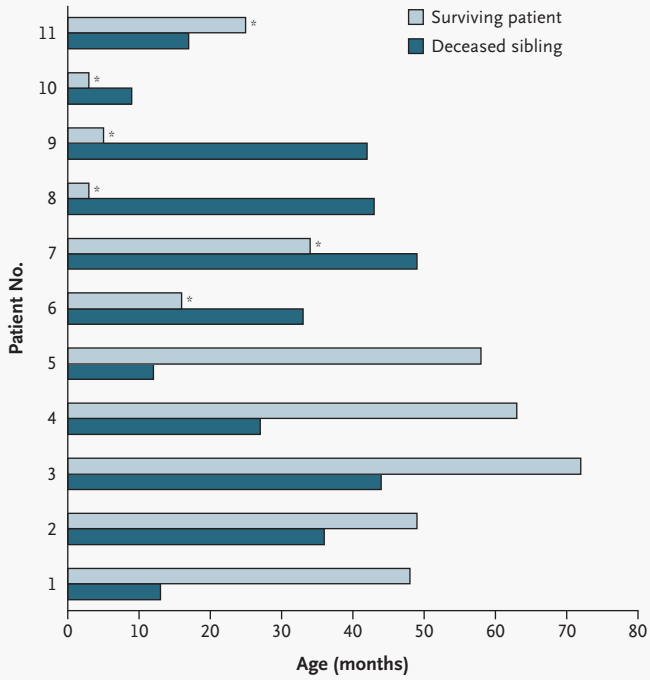

Figure 2. Survival of Patients and Siblings.

The age at death of untreated siblings of patients with Krabbe's disease who underwent transplantation is shown, as is the age of newborns who underwent transplantation and who were alive as of January 28, 2005. Six patients had outlived their siblings by 8 to 48 months. An asterisk indicates patients who have not yet reached the age of death of their siblings.

Electroencephalography

EEGs were available for all patients. Eight of the newborns had normal results both before and four months to six years after transplantation. One patient had a normal EEG before transplantation at the age of 1 month, but EEG examination at 6.5 months of age showed excessive delta activity during a nap. Subsequent EEGs were normal. Another patient showed temporal sharp waves before transplantation, at 10 days of age, with no subsequent tests performed to date. One patient's pretransplantation EEG was abnormal at one month of age, showing sharp waves and asymmetric delta activity, and was not repeated. No patients had clinical seizures.

In the symptomatic group, all patients had ab-

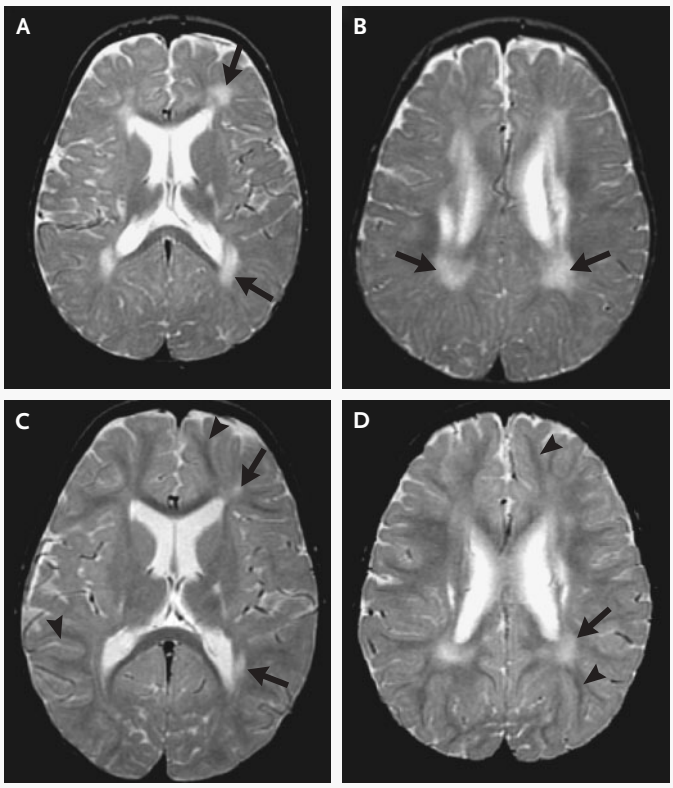

Figure 3. Serial MRIs of a Newborn Transplant Recipient One Year (Panels A and B) and Two Years (Panels C and D) after Transplantation.

Serial MRI scans show the progression of myelination and the decrease in the size of abnormal regions of white matter in a girl who underwent transplantation at three weeks of age. An axial T_2-weighted image through the level of the corpus callosum, obtained when the patient was one year of age (Panel A), shows an abnormal hyperintense signal in the periventricular regions (arrows) and along the posterior limb of the left internal capsule. A normal hypointense signal consistent with normal myelination is seen within the corpus callosum. An axial T_2-weighted image through the centrum semiovale obtained at one year of age (Panel B) also shows an abnormal hyperintense signal in the periventricular regions (some of which are indicated by arrows). Normal myelination is seen within some regions of white matter lateral to the areas of abnormal signal intensity. In Panel C, an axial T_2-weighted image through the same level as shown in Panel A, obtained at two years of age, shows a marked reduction in size of the periventricular regions of an abnormal hyperintense signal (arrowheads) and the development of a normal hypointense signal in the peripheral white matter (arrows), consistent with the progression of normal myelination. In Panel D, an axial T_2-weighted image through the same level as shown in Panel B, obtained at two years of age, shows a reduction in the size of the periventricular regions of an abnormal hyperintense signal (arrowheads). Progression of normal myelination is seen within the regions of peripheral white matter (arrow).

normal EEGs before and up to three years after transplantation. All surviving patients had clinical seizure activity at the most recent follow-up examination.

NEURODEVELOPMENTAL FUNCTION

Ten of the 11 patients in the newborn group were evaluated after transplantation. In the symptomatic group, 8 of the 14 patients were evaluated in all domains. None of the symptomatic patients improved appreciably in any area (Fig. 4A).

Cognitive Function

The 10 newborns whom we evaluated continued to gain cognitive skills at a normal rate (Fig. 4A). However, two patients scored below normal in some of the subtests because of difficulties with fine motor control.

Adaptive Behavior

Adaptive behavior is a standardized measure of independent and self-help skills and is based on parents' perceptions of their infant's abilities. Eight newborns were within the average range and two were below average when most recently tested at 6 months to 5.5 years after transplantation (Fig. 4B).

Language

All but one newborn had normal receptive language (the ability to understand communication through gestures, facial expressions, and words) (Fig. 4C). Expressive language (the ability to express needs with the use of gestures, vocalization, facial expressions, and words) was below average in two patients (Fig. 4D). Articulation difficulties secondary to motor involvement ranged from mild to severe and accounted for the delay in expressive language.

Gross Motor Function

Before transplantation, four of the asymptomatic newborns had subtle motor abnormalities such as a weak sucking reflex, a poor rooting reflex, hypotonia, and hypertonia. The other seven babies appeared normal.

Post-transplantation evaluations of gross motor skills occurred in 10 newborns at 4 to 66 months of age (Fig. 4E). Of the 10 children, 4 had mild-to-severe delays in the development of gross motor skills. Two of the four had subtle motor abnormalities at birth, and two appeared normal. One continued to have severe delays at 33 months.

The remaining six patients gained gross motor skills during the first year of life. During the second and third years of life, progressive spasticity in the lower extremities and truncal weakness developed in two of the six children, who had initially pulled up to stand, which prevented them from walking

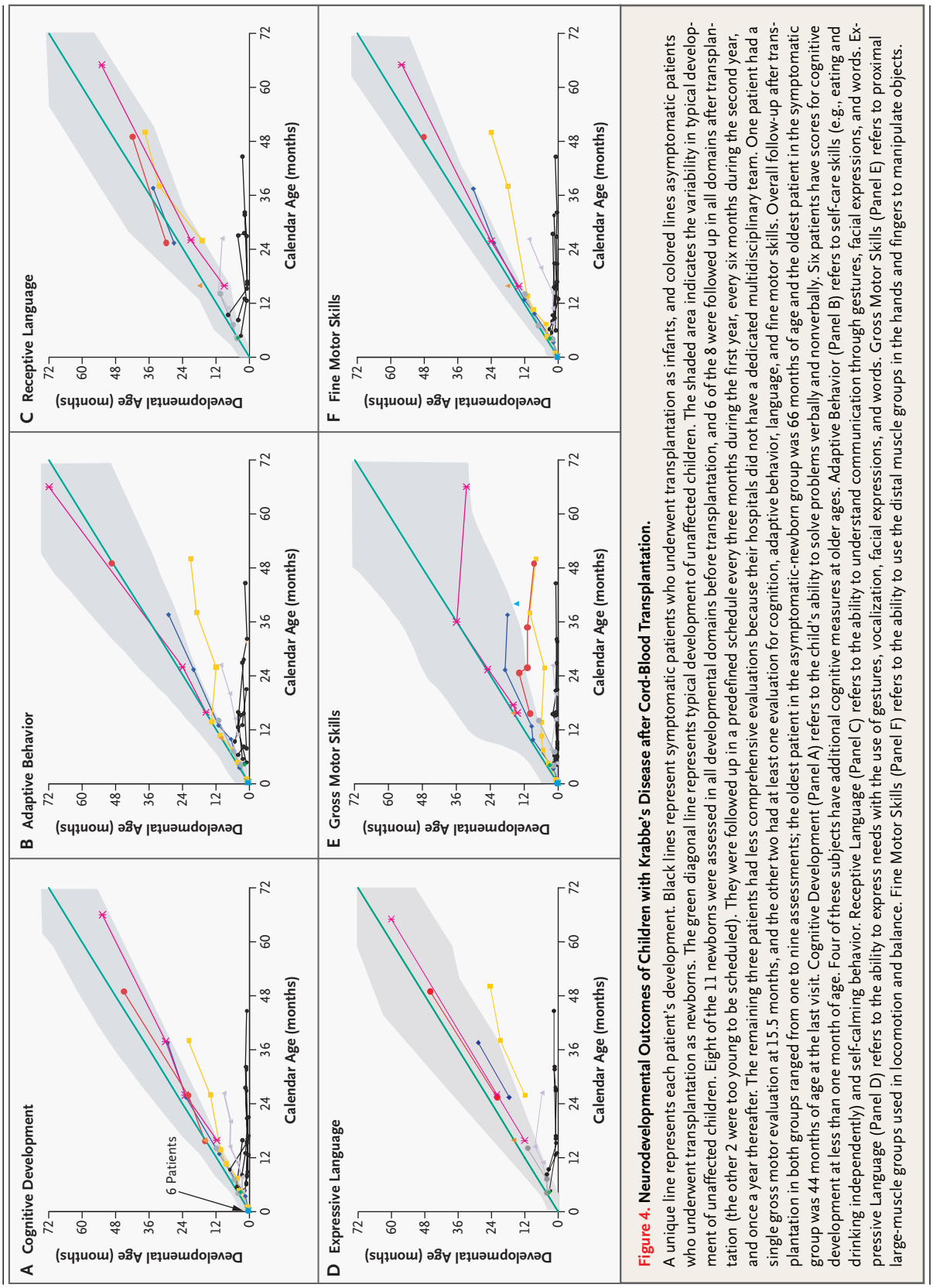

Figure 4. Neurodevelopmental Outcomes of Children with Krabbe's Disease after Cord-Blood Transplantation.

A unique line represents each patient's development. Black lines represent symptomatic patients who underwent transplantation as infants, and colored lines asymptomatic patients who underwent transplantation as newborns. The green diagonal line represents typical development of unaffected children. The shaded area indicates the variability in typical development of unaffected children. Eight of the 11 newborns were assessed in all developmental domains before transplantation, and 6 of the 8 were followed up in all domains after transplantation (the other 2 were too young to be scheduled). They were followed up in a predefined schedule every three months during the first year, every six months during the second year, and once a year thereafter. The remaining three patients had less comprehensive evaluations because their hospitals did not have a dedicated multidisciplinary team. One patient had a single gross motor evaluation at 15.5 months, and the other two had at least one evaluation for cognition, adaptive behavior, language, and fine motor skills. Overall follow-up after transplantation in both groups ranged from one to nine assessments; the oldest patient in the asymptomatic-newborn group was 66 months of age and the oldest patient in the symptomatic group was 44 months of age at the last visit. Cognitive Development (Panel A) refers to the child's ability to solve problems verbally and nonverbally. Six patients have scores for cognitive development at less than one month of age. Four of these subjects have additional cognitive measures at older ages. Adaptive Behavior (Panel B) refers to self-care skills (e.g., eating and drinking independently) and self-calming behavior. Receptive Language (Panel C) refers to the ability to understand communication through gestures, facial expressions, and words. Expressive Language (Panel D) refers to the ability to express needs with the use of gestures, vocalization, facial expressions, and words. Gross Motor Skills (Panel E) refers to proximal large-muscle groups used in locomotion and balance. Fine Motor Skills (Panel F) refers to the ability to use the distal muscle groups in the hands and fingers to manipulate objects.

or standing independently. These children, at 63 and 58 months of age, were able to sit independently, stand with assistive devices, and ride adaptive tricycles. Another patient with gross motor abnormalities had bilateral hip dysplasia at 48 months of age, which complicated her gross motor development, but she was able to walk independently with a walker. Two patients, now five and seven years of age, developed normally until two and three years of age, respectively. Both can walk, run, and jump independently, but neither has acquired more sophisticated gross motor skills (e.g., skipping, balancing, and hopping on one leg). The remaining five children evaluated at 4, 6, 7, 13, and 16 months, respectively, continue to gain gross motor skills appropriately. The patient who was not evaluated after transplantation is able to walk. All the surviving patients in the symptomatic group are severely affected with a developmental level equivalent to that of a one-month-old.

Fine Motor Function

Of the 10 newborns tested between 4 and 66 months of age, 8 had average fine motor skills and 2 had severe delays in the development of these skills (Fig. 4F). Of the two newborns with delayed development of fine motor function, one had a tendency to pronate the arms and the other to clasp the thumbs. One older patient has not been tested. All surviving patients in the symptomatic group are severely impaired and cannot manipulate objects with their fingers.

Growth

All patients were smaller than average for height, and some for weight (Fig. 5A through 5D). In contrast, head circumference measurements were within 2 SD of normal in patients in both the asymptomatic-newborn group and the symptomatic-infant group (Fig. 5E and 5F).

DISCUSSION

We evaluated the feasibility, safety, and efficacy of cord-blood transplantation from unrelated donors in 11 asymptomatic newborns and 14 symptomatic infants with infantile Krabbe's disease. In the newborn group, a family history of the disease permitted early diagnosis and treatment before the onset of clinical symptoms. Radiation therapy was avoided because of the known adverse late effects. All newborn patients who had engraftment survived and, as of January 28, 2005, had durable donor chimerism and normal peripheral-blood enzyme activity. The one patient who did not receive antithymocyte globulin continued to have stable mixed donor–recipient chimerism. In contrast to untreated patients, who had overwhelming spasticity, blindness, and early death by one to two years of age, the newborns who underwent transplantation before the onset of symptoms maintained normal vision and hearing and normal cognitive development except for areas influenced by gross motor development. Some have continued to gain gross motor skills. Infants who underwent transplantation after the development of symptoms had a higher rate of death and minimal neurologic benefits from transplantation.

Cord-blood units from unrelated donors with matching at four to six of six HLA loci were selected, rather than bone marrow from unrelated adult donors, in order to permit patients to undergo transplantation in the shortest possible time. We also hypothesized that cord blood may contain a younger population of stem cells capable of tissue repair and regeneration. Because of the known polymorphisms affecting blood galactocerebrosidase activity,[22] multiple donors were screened for each patient to select a donor with high levels of enzyme expression. Patients maintained enzyme levels within the range of the level measured in the cord-blood unit before its selection for transplantation. Because of their small size, all patients underwent transplantation with very high doses of nucleated cells per kilogram of body weight. This resulted in faster engraftment than in older patients who underwent transplantation with cord blood.[23]

In previous reports, bone marrow transplantation in mildly symptomatic patients with a later onset of Krabbe's disease has arrested disease progression, facilitated myelination, and reversed neurologic deficits.[2] In the present study, cord-blood transplantation in symptomatic patients from 4 to 11 months of age resulted in some stabilization of neurologic disease, but the surviving patients remain severely impaired. In these babies, irreversible damage to motor tracts probably preceded the intervention with transplantation. In contrast, we showed that the patients who underwent transplantation as newborns had substantial neurologic benefits and developmental gains, including increased myelination on serial brain MRI scans and,

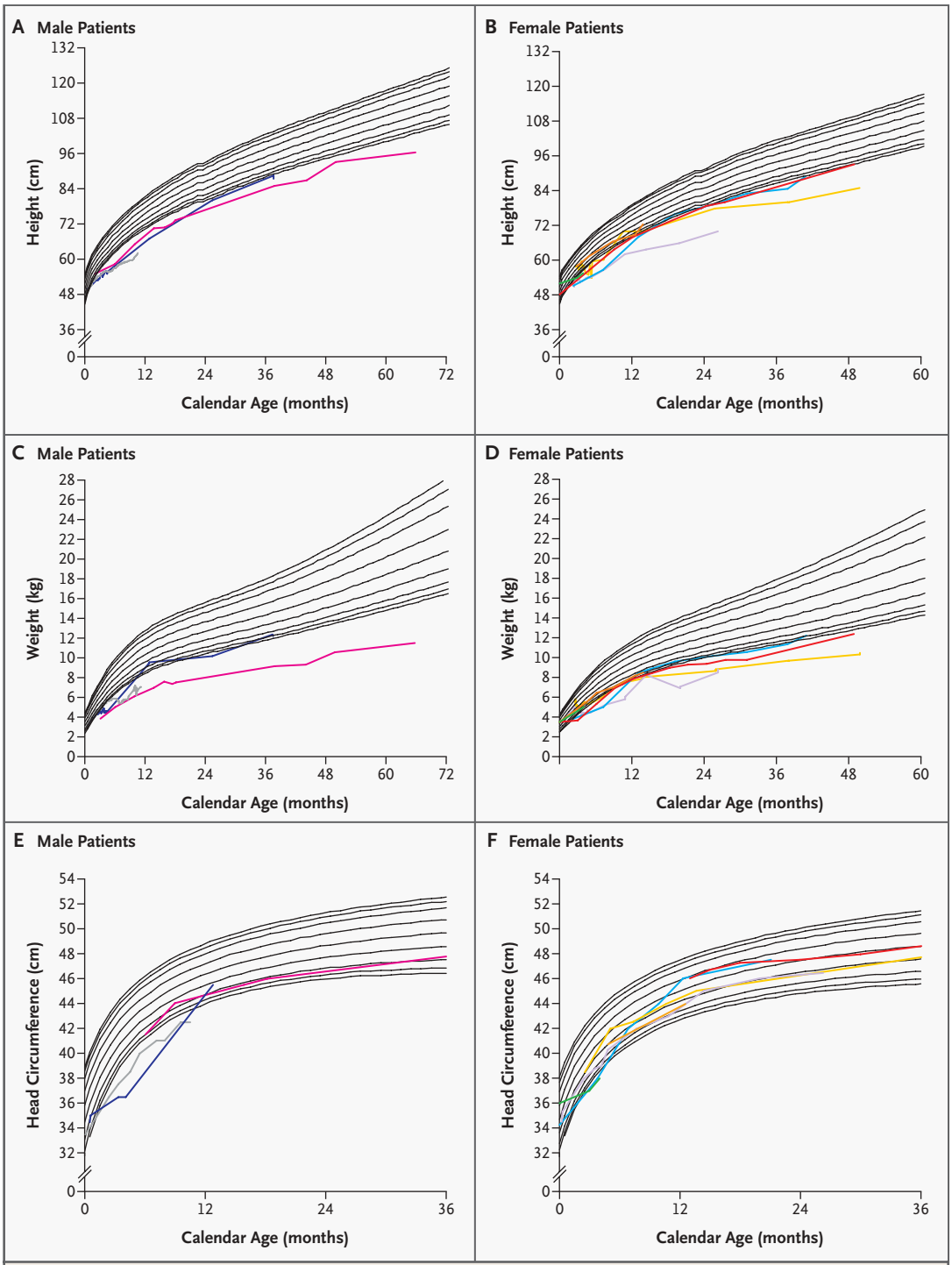

Figure 5. Growth According to Sex from Birth to the Age of 36, 60, or 72 Months in Asymptomatic Newborns.

Growth for height, weight, and head circumference was plotted for male and female patients who underwent transplantation during the newborn period. The black curves represent standard growth curves (3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th percentiles). The colored lines represent individual patients. Height was at or below the fifth percentile, weight was below the fifth percentile, but head circumference remained in the normal range in all but one patient.

in some patients, improvement in nerve-conduction studies. Vision, hearing, and cognitive abilities were preserved.

Despite the substantial neurodevelopmental gains in the newborns, some degree of deficit in gross motor function became apparent in all the children. Variable motor function, from nearly normal to an inability to walk without assistance, may be attributed to different rates of central myelination. For example, there were patients in whom gross motor function was not progressing, whereas fine motor function continued to develop appropriately, suggesting that motor areas that have myelination early in childhood were affected more than those that are myelinated later.

Some babies may also have had irreversible damage prenatally or in the first few weeks after birth. In others, disease progression may have been slower, permitting rescue of the motor tracts by transplantation. Although definite progression of myelination was seen on MRI, the results of nerve-conduction studies improved substantially in only a subgroup of patients, suggesting that the effects of cord-blood transplantation on myelination may differ in the central and peripheral nervous systems. This hypothesis is supported by previous studies in the "twitcher" mouse (an animal model of globoid-cell leukodystrophy) that show a similar discrepancy in the correction of central and peripheral disease after bone marrow transplantation.[24,25]

The results in the 11 newborns in whom Krabbe's disease was diagnosed prenatally or at birth show indisputable benefits with minimal morbidity, despite an aggressive approach. For reasons yet to be explained, cognitive function is preserved despite motor impairment. It is possible that the transplant will delay but not prevent eventual neurologic decline or that the early decline in motor function will stabilize over time. The long-term neurodevelopmental course of these patients can be determined only with further follow-up. The advent of more sensitive neuroimaging technology may clarify the stages of damage in newborns with Krabbe's disease, thus permitting correlations of early studies with outcomes.

The results of this study show that transplantation of umbilical-cord blood from unrelated donors in newborns with Krabbe's disease is associated with substantially better neurologic outcomes and survival than is no therapy[21] or transplantation after symptoms develop. The marked differences in outcome when transplantation is performed in asymptomatic newborns and when it is performed in older symptomatic infants have implications for decisions regarding the implementation of newborn-screening programs for lysosomal storage diseases.

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