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Benefits of Newborn Screening and Hematopoietic Cell Transplant in Infantile Krabbe Disease

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Abstract:
Infantile Krabbe Disease (IKD) can be treated with hematopoietic cell transplantation (HCT) if done during the first weeks of life before symptoms develop. To facilitate this, newborn screening (NBS) has been instituted in eight U.S. states. An application to add KD to the Recommended NBS Panel (RUSP) is currently under review. In this report, the outcomes of newborns with IKD diagnosed through NBS and treated with HCT are presented. The unique challenges associated with NBS for this disease are discussed including opportunities for earlier diagnosis and streamlining treatment referrals. This is a retrospective review of infants with IKD detected by NBS and referred for HCT. The timing from diagnosis to HCT were examined and both HCT and neurodevelopmental outcomes are described. Six infants were diagnosed and referred for HCT. Neurologic testing before HCT revealed evidence of active IKD in all infants. All underwent HCT between 24-40 days of age, successfully engrafted, and are alive 30-58 months later (median, 47.5 months). All are gaining developmental milestones albeit at a slower pace than unaffected age-matched peers. Gross motor function is most notably affected. NBS for these patients enabled early access to HCT, the only currently available treatment for infants with IKD. All children are alive and have derived developmental and neurologic benefits from timely HCT. Long-term follow up is ongoing. Optimization of HCT and further development of emerging therapies, all of which must be delivered early in life, are expected to further improve outcomes of infants with IKD.

Conflict of interest: COI declared - see note

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Abstract.

Infantile Krabbe Disease (IKD) can be treated with hematopoietic cell transplantation (HCT) if done during the first weeks of life before symptoms develop. To facilitate this, newborn screening (NBS) has been instituted in eight U.S. states. An application to add KD to the Recommended NBS Panel (RUSP) is currently under review. In this report, the outcomes of newborns with IKD diagnosed through NBS and treated with HCT are presented. The unique challenges associated with NBS for this disease are discussed including opportunities for earlier diagnosis and streamlining treatment referrals. This is a retrospective review of infants with IKD detected by NBS and referred for HCT. The timing from diagnosis to HCT were examined and both HCT and neurodevelopmental outcomes are described. Six infants were diagnosed and referred for HCT. Neurologic testing before HCT revealed evidence of active IKD in all infants. All underwent HCT between 24-40 days of age, successfully engrafted, and are alive 30-58 months later (median, 47.5 months). All are gaining developmental milestones albeit at a slower pace than unaffected age-matched peers. Gross motor function is most notably affected. NBS for these patients enabled early access to HCT, the only currently available treatment for infants with IKD. All children are alive and have derived developmental and neurologic benefits from timely HCT. Long-term follow up is ongoing. Optimization of HCT and further development of emerging therapies, all of which must be delivered early in life, are expected to further improve outcomes of infants with IKD.

Key Points

- Expanded newborn screening identified six infants with infantile Krabbe Disease; All underwent transplant before six weeks of age.
- All infants engrafted and survived HCT. Now 30-58 months old, neurodevelopmental gains continue slowly with prominent gross motor delays.

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Krabbe disease (KD) is a fatal, inherited lysosomal storage disorder caused by deficiency of β-galactocerebrosidase (GALC) with resulting increases in psychosine (galactosylsphingosine) leading to aberrant central and peripheral nervous system myelination. Disease manifestations are most severe in the infantile (IKD) form. Initially appearing healthy, affected infants experience rapid neurologic decline leading to death at a median of two years of age[1]. Low GALC activity coupled with a high psychosine level (a prognostic biomarker) support a neonatal diagnosis of IKD[2-4].

Hematopoietic cell transplantation (HCT), performed emergently ideally before 30 days of age, has a survival and functional benefit in pre-symptomatic infants with IKD[5-7]. To enable this, New York (NY) initiated newborn screening (NBS) for KD over 15 years ago.[3,8,9] Since then, approximately 3.5 million infants have been screened leading to the diagnosis of six infants (including two biological siblings) with IKD. This incidence was lower than originally predicted and the outcomes of four infants transplanted were less favorable than those diagnosed *in utero* or at birth due to family history.[3,7-9] An additional eight states now screen newborns for KD: Illinois[10], Indiana, Kentucky, Missouri, New Jersey, Ohio, Pennsylvania, and Tennessee. Three additional states (Georgia, South Carolina and Louisiana) plan to begin screening soon. To date, nearly 6 million infants have been screened. Six additional infants screened positive and were subsequently diagnosed with IKD in states other than NY over the past 5[11]. In this report, we describe the outcomes of these six infants identified through NBS and who underwent HCT for IKD. These patients illustrate the successes and challenges of NBS for IKD.

Methods. This retrospective study represents a collaboration between four experienced pediatric HCT centers: Duke Children's Hospital (coordinating center; Durham, NC), Ann & Robert H. Lurie Children's Hospital (Chicago, IL), Nationwide Children's Hospital (Columbus, OH) and St. Louis Children's Hospital (St. Louis, MO). All centers had prior experience in transplanting children with metabolic diseases and one center (Duke) had previously transplanted infants with IKD. Infants included here were born between January 2016 and February 2019. To our knowledge, this report includes all infants diagnosed with IKD by NBS programs outside of NY state. Infants born in NY have been previously reported[3,8,9]. Following Institutional Review Board approval, data was retrospectively collected from electronic medical records and entered into a secure, HIPAA-compliant RedCap (Research Electronic Data Capture) database[12] hosted at Duke University. The study was conducted in accordance with the Declaration of Helsinki. The primary study endpoint was the evaluation performed 1 year after HCT. Descriptive endpoints including clinical, neurodiagnostic, and neurodevelopmental outcomes were included with follow-up ranging from 30-58 months.

Newborn Screening and Referral. NBS programs followed state-specific algorithms for testing, diagnosis and referral of infants with IKD which beyond the scope of this current report.[3,13] Low GALC activity measured on standard dried blood spots triggered reflex testing, the details of which varied by state. Infants met state-specific criteria and were referred for emergent HCT evaluation at a participating center. Five infants were evaluated as inpatients to expedite the process. Prior to transplant, the diagnosis was confirmed through low GALC activity in leukocytes, elevated psychosine and/or GALC genotyping. Genotyping testing was performed in all infants, but the results were not always available to aid in the decision to proceed to transplant.

Neurologic Evaluations. Detailed neurologic evaluations were performed before HCT, and at timepoints determined by institutional practices including 6-months, 12-months and annually. Evaluations included detailed physical exams by child neurologists, neurodevelopmental testing, magnetic resonance imaging (MRI), nerve conduction studies (NCS), brainstem auditory-evoked responses (BAER), visual evoked potentials (VEP), and electroencephalograms (EEG). Lumbar punctures were performed to measure protein in cerebral spinal fluid (CSF). Three patients enrolled in a separate research study measuring CSF psychosine[14].

Neurodevelopmental testing was scored by neuropsychologists at the treating institution. Testing utilized the Bayley Scales of Infant and Toddler Development, Third Edition (BSID-III), a comprehensive norm-referenced assessment that provides composite scores (mean: 100, standard deviation (SD): 15, range 40-160) in Cognitive, Language, and Motor developmental domains[15]. Five subscales provide Cognitive, Receptive Language, Expressive Language, Fine Motor and Gross Motor development scores (mean: 10, SD:3, range 1-19). Growth scores (mean: 500, SD: 100, range 200-800) were calculated to reflect longitudinal growth independent of age[15].

Neuroimaging and neurophysiologic testing protocols were per institutional standards. Brain MRIs were interpreted by pediatric neuroradiologists at the treating institution. NCS data was compared to age-based normative values for latency (marker of demyelination) and amplitude (marker of axonal neuropathy) in upper and lower extremity motor and lower extremity sensory nerves[16]. BAERs and VEPs were scored abnormal per established guidelines if the I-V interpeak latency was prolonged or if P1 waveforms were absent.[17-19] EEG results were scored as: normal, abnormal (generalized slowing or discharges present), or "excessive sharp transients' (ESTs). The latter designation was maintained due to disagreement in the field regarding the significance

of ESTs[20,21]. Brain MRIs and neurodiagnostic testing was independently reviewed by neuroradiologists (A.M., M.H. J.P.) or neurologist (M.R.), respectively.

***Transplantation*.** Infants underwent standard evaluations to assess health, infectious disease screening, organ function, and clinical status of IKD, similar to published guidelines[22,23]. Informed consent for HCT was provided by all parents/legal guardians and palliative care without HCT was offered as an alternative path. Cord blood donors were selected from the Be the Match® registry using standard criteria, and, when possible, GALC enzyme activity was measured on attached cryopreserved aliquots to avoid selecting carrier donors[22]. Central venous lines were placed prior to HCT. Five infants received gastrostomy tubes to administer medications and nutrition. Chemotherapy could start before final donor selection if (1) multiple donors were tested to ensure at least 1 suitable donor was confirmed and available on the day of transplant and (2) the National Marrow Donor Program had given approval. Myeloablative busulfan (adjusted per pharmacokinetics), cyclophosphamide 200 mg/kg (with mesna chemoprotection) and anti-thymocyte globulin (total dose 90 mg/kg) were administered over nine days followed by donor cell infusion the next day (HCT day 0). Graft-versus-host disease prophylaxis was cyclosporine or tacrolimus with mycophenolate mofetil. Two infants were co-enrolled on a clinical protocol testing an adjunctive intrathecal administration of UCB-derived oligodendrocyte-like cells 4-6 weeks post-transplant (Patients 1-2; NCT02254863; IND#15338)[24].

Statistical methods. Descriptive statistics were calculated for clinical and neurodevelopmental outcomes. BSID-III standardized and growth scores allowed for comparison to age-based norms and to follow longitudinal development, respectively.[15] Standard definitions were used for neutrophil and platelet engraftment.[25] Full donor chimerism was defined as >95% donor cells in

both myeloid and lymphoid lineages.[25] Acute and chronic graft-versus-host disease was graded according to consensus criteria.[26,27]

Results.

Newborn screening and referral. NBS identified six infants (four males, two females) with IKD. All had negative family histories and one infant had (two) full unaffected siblings. Families were notified of the abnormal NBS results at a median of 6 days of age (range, 5-16; Figure 1). One infant needed an additional blood drawn for confirmatory testing at postnatal day 9 which resulted 7 days later.

All infants, initially identified by low GALC enzyme levels, had extremely elevated psychosine levels (range, 24-73 nmol/L, normal <2 nmol/L) confirming the IKD diagnosis (Table 1). *GALC* genotyping revealed known pathogenic variants in five infants (Table 1). The 30-kB deletion, which is associated with severe phenotype and European or Mexican ancestry, was detected in three infants [1 homozygous (Northern European White); 2 heterozygous (both multiracial)][28]. One infant (Patient 2) had novel mutations predicted to severely disrupt GALC synthesis. This infant's very high psychosine supported the decision to proceed to HCT.

Pretransplant evaluations. Infants were 7-21 days old when initial HCT consultations occurred (Figure 1) followed immediately by pre-HCT evaluations lasting 6-15 days. All infants demonstrated abnormalities on at least one neurodiagnostic test performed before transplant (maximum: 4 abnormal tests; Table 1). Brain MRIs were abnormal in three infants. The three infants with normal MRIs had abnormalities noted on 2-3 other neurodiagnostic tests before HCT. NCS and BAER studies were abnormal in five infants and not done in one. VEP studies were abnormal (n=2), normal (n=2) or not performed (n=2). Four of six infants had ESTs on

EEG. CSF protein was elevated in four infants (range, 332-444 mg/dL), normal in one (117 mg/dL; normal 30-200 mg/dL) and not performed in one. Three infants were co-enrolled in a research study measuring CSF psychosine and all three had elevated levels[14]. All had normal organ function and infectious disease screening. On initial physical examination, one infant had axial hypotonia and two had "cortical thumbs".

Transplantation outcomes. Chemotherapy started at a median of 27 days old (range, 14-32; Figure 1). Busulfan (oral n=4; intravenous n=2), cyclophosphamide and anti-thymocyte globulin (equine n=5; rabbit n=1) were administered without adverse events. Infants were a median 36 days old (range, 24-40 days) on the day of HCT. Donor-cells engrafted, reaching an ANC of 500/uL between days 12-24 post-HCT (Table 2). All infants are alive with normal GALC levels with a median of 47.5 months follow-up (range, 30-58). Two patients have mixed chimerism at last follow up but with normal enzyme and low psychosine levels compared to baseline.[25] Five children have normal immune function and require no transplant-related medications. One child (patient 2), who is currently 58 months post-HCT, has needed intermittent immunosuppression for autoimmune hemolytic anemia.

Neurologic outcomes. The children (30-58 months old) have varying degrees of developmental delay (Supplemental Table 1). All take some nutrition by mouth and three receive supplemental gastrostomy-tube feeds. Five independently drink from cups, four feed themselves crackers using a pincer grasp and two use utensils. Each can hold, transfer, and manipulate objects including playing games on tablets/smartphones. One child has >75 words and uses 1-2-word phrases Another child has 15-20 words and >75 signs; the remaining children use single words or approximations along with signs/gestures. Two uses an augmentative and alternative communication (AAC) device. All have varying degrees of lower extremity spasticity and

weakness that limits ambulation and impacts adaptive functioning. One walks independently and can climb. All can sit unsupported and the other five children have some form of locomotion (limited walking with gait trainer, cruising, or crawl/roll for locomotion). Four use self-propelling wheelchairs. Generally, upper body motor function is less impaired than the lower body. Anecdotally, the two infants enrolled on the experimental protocol (as described in the methods) did not appear to derive any additional clinical benefit from the treatment.

Neurodevelopmental outcomes. Five infants underwent neurodevelopmental testing 1 year after HCT. BSID-III composite scores were similar among the five infants (Figure 2A): cognitive (range, 75-80), language (range, 65-83) and motor (range, 46-77). All scores were >1 SD below the mean using aged-based norms (mean: 100, SD: 15, range 45-160). Similarly, subscale scores were all below the mean (10; SD: 3; range 1-19; Figure 2B). In general, the children demonstrated stronger cognitive (range, 4-6), receptive (range, 3-8) and expressive language (range, 3-7) skills compared to motor skills. Fine motor skills (range, 1-7) varied between infants whereas gross motor scores were uniformly extremely low. Over time, they steadily gained milestones across all domains (Figure 2C-G) although the pace was slower than typically developing children. The remaining child (Patient 6) has generally been lost to follow-up and neurocognitive testing has not been completed.

Neurodiagnostic testing. All infants underwent a variety of neurodiagnostic testing (performed per local protocols) to determine the extent of disease. Three infants had normal MRIs before transplant. One MRI was normal at 6-months. All MRIs were abnormal by 1 year after transplant and remain so at last follow-up. NCS were uniformly abnormal at all timepoints (Figure 3A-E). Mixed demyelinating and axonal neuropathies (sensory and motor) was present from birth in the infants tested and worsened over time. In the neonatal studies, sensory action potentials proved

challenging to measure due their smaller signals. Only 1 of 9 nerves tested had a recordable signal. Sensory nerve data ranged from 20 to 70% of normal values. Six nerves (of the 9 tested) were reactive in the upper and/or lower extremity motor studies but all were abnormal. Motor neuropathy was present in neonates with approximately 30-75% of normal values of amplitude and velocity. Motor neuropathy stabilized at a severely affected level with up to 33 months of follow up. Taken together, the NSC findings indicate persistent mixed sensory motor demyelinating and axonal neuropathy that is progressive over time in most patients. Of note, neuropathy may be confounded by chronic steroid use and other HCT related drugs. Five infants had abnormal BAERs before HCT (one not tested; Figure 3G, Supplemental Table 2. One infant's BAER temporarily normalized at six months, but all were abnormal at 1- and 2-years. Two of five infants had abnormal VEP studies before HCT and one remained abnormal over time. EEGs were initially normal (n=2) or demonstrated ESTs (n=4; Supplemental Table 2). Three of four infants developed background slowing after HCT. None have developed seizures to date. GALC enzymes levels normalized after HCT (range, 0.6-2.1 nmol/hr/mg; normal >0.5), whereas blood psychosine levels decreased but did not normalize after transplant (Supplemental Table 2).

Discussion.

In this report, we present the outcomes of six newborns diagnosed with IKD through expanded NBS in their states. Early diagnosis of IKD enabled access to emergent HCT, the only currently available treatment for infants with this disease. The newborns appeared healthy, yet all demonstrated evidence of active IKD on neurodiagnostic and neuroimaging studies performed before transplant (at 1-3 weeks of age), highlighting the rapid disease course. The newborns tolerated myeloablative chemotherapy well, engrafted donor cells, and established normal GALC

levels. The children, now 30-58 months old, are all alive and all but one patient are off immunosuppressive therapy. One child has intermittent transplant-related autoimmune hemolytic anemia treated with low-dose immunosuppression[29]. Notably, all children are continuing to achieve developmental milestones, albeit slower than unaffected peers. HCT itself has been associated with developmental delays in pediatric patients[30], but the impact of HCT here is not quantifiable. In contrast, untreated infants develop quadriparesis, severe motor delay (equivalent to 1-month old), autonomic instability by 1-year of age and early death[1,31]. This report highlights that children with IKD can benefit from early HCT with improved survival and improved neurological function although varying degrees of neurologic impairments remain.

Testing newborns for IKD presents unique challenges compared to other diseases diagnosed by NBS. For infants to access HCT or other future treatments, complex care coordination is emergently needed and, for 2 of 6 infants, this included a referral to an out-of-state transplant center. Prior reports established HCT before 30 days old as a benchmark among pre-symptomatic infants[5]. Only one infant achieved that goal here. The family was integrally involved in hastening the referral, thereby allowing early evaluation (7-days old) and transplant (24-days old). Comparatively, the other babies were 31-40 days of age at HCT. Due to the small numbers, we cannot comment on whether this timing impacted the outcomes. Taken together, our experience reflects real-world practice. NBS programs in this series encountered reflex testing delays (e.g., need for extra blood draw), difficulties contacting the family, and delays communicating results. Kwon *et al* found sources of delays in the NY series included using genotyping as a confirmatory test and consulting local specialists rather than referring directly to a transplant center[3]. These steps were eliminated in this patient cohort. Possible remedies include: (1) rapid reflex testing using psychosine per recent consensus recommendations[3]; (2)

established referral pathways triggered by reflex testing; and (3) concurrent pathways to rapidly inform the pediatrician and initiate the referral. When NBS is implemented, relationships with transplant centers should be firmly established. Strategies for transplant centers to mitigate delays include: (1) performing transplant-related processes and disease evaluations in parallel; (2) partnering with Medicaid and private healthcare payers to minimize third-party payer approval time; and (3) initiating chemotherapy before final donor selection assuming candidate donors have been selected (not customary for HCT centers). It is unknown if gene therapy, which is still in developmental stages, can reverse or prevent further neurologic damage in these patients but pre-clinical animal data also demonstrates a need for treatment in the first few weeks of life to optimize outcomes. Presently, HCT will continue to be an emergent option for patients with IKD. Clearly, opportunities for improvement still exist for both NBS programs and treating centers to maximize efficiency.

Transplant after onset of clinical symptoms yields very poor outcomes[7]. In pre-symptomatic infants, early and long-term neurologic and transplant outcomes have been published[5-8]. Escolar *et al* described 11 infants diagnosed by family history within a larger cohort of 25 infants.[7] To facilitate early HCT, most mothers delivered at the transplant center and often were induced 2-4 weeks early. Nine of the 11 are alive with varying degrees of motor disability after a median of 18 years.[32] One died after an anesthesia reaction and another died of progressive IKD (5 and 15 years, respectively). One decade later, the NY state NBS program published their experience[8]. Four infants underwent HCT between 24-41 days old using similar chemotherapy as reported here. Of note, supportive care measures have since improved. Two infants died shortly after HCT. Two are long-term survivors (14- and 10- years): one has moderate impairment (attends school with educational supports; converses; uses wheelchair; HCT at 32 days old) and one has

severe neurologic impairment (full care; HCT at 41 days old)[8], raising concerns of HCT efficacy by Ehmann and Lantos[33]. Comparatively, infants diagnosed by family history experienced more favorable outcomes although this should be interpreted cautiously given the small numbers. Ehmann and Lantos postulated that many of these infants were "false positives" (i.e., healthy), but as noted by Orsini *et al*, this is unlikely based on long-term outcomes[6,34]. Most were diagnosed prenatally and delivered at the HCT center thereby allowing evaluations to quickly begin. Logistics such as housing, sibling childcare and financial approvals were arranged ahead of time. With their prior experiences, affected families may have found the decision to proceed to transplant easier.

One challenge has been distinguishing true- from false-positive newborn screens (i.e., low GALC). While GALC enzyme activity has merits as a screening test, it lacks the sensitivity needed to differentiate affected from unaffected babies, a concern raised by Ehmann and Lantos[33]. PCR-based deletion analysis and *GALC* sequencing was previously used as a second-tier test[9,35], although is labor intensive, expensive, and can potentially miss novel pathogenic variants (e.g., Patient 2) or provide indeterminate results (variants of uncertain significance). As such, the biomarker psychosine has become the reflex test of choice. It can rapidly discriminate between IKD ($\geq$10 nmol/L), late-onset forms (>2 and<10 nmol/L), pseudo-deficiencies and carriers (<2nmol/L)[2]. Providing further evidence for psychosine-based confirmatory testing, all six infants had markedly elevated psychosine levels on their newborn dried blood spots. Most states screening for Krabbe Disease have now incorporated psychosine into reflex testing algorithms, but this was only partially implemented when the infants in this study were identified. Several programs now perform psychosine testing "in-house", and others have since arranged for faster sample turnaround. Going forward, samples could ideally be screened,

undergo reflex psychosine testing, and have results between day 2-4 of life (depending on in-house vs. referral laboratory). We have demonstrated that, with established referral protocols, it is feasible to begin evaluations even the next day (days 3-5 of life). Once the diagnosis and disease activity is confirmed, chemotherapy can start shortly thereafter assuming donor selection is nearly complete. This timeline, enabled by psychosine reflex testing and other strategies outlined above, could allow for HCT to occur when the infant is 17-20 days old.

This report represents the largest series of infants with IKD diagnosed through NBS. Rapid diagnosis using psychosine levels on newborn blood spots enabled early referrals and transplantation before 6 weeks of age. The children in this series are alive and have derived neurologic benefits from HCT compared to untreated children. Nevertheless, all have ongoing developmental delays and motor disabilities. This report adds considerably to what is known about modern outcomes after HCT in newborn infants with IKD and will inform ongoing conversations about widespread NBS for KD. It also demonstrates the feasibility of treatment at centers closer to the families' homes, but the urgent timeline does introduce challenges. Beyond the HCT recovery period, children generally receive their long-term care locally and are seen at larger pediatric centers 1-2 times per year. NBS enabled early access to HCT for these patients, but as new therapies emerge, it is likely early treatment (as neonates) will needed to optimize outcomes. Thus, in combination with expanded NBS to identify affected infants, efforts to optimize HCT and develop new treatments are ultimately needed.

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Legends.

Figure 1. Timelines for Patient Diagnosis, Referral and Transplant. Timelines are presented for each infant showing when: (1) families were first alerted to abnormal newborn screening (NBS; date indicated by *), (2) date (indicated by †) when NBS results were finalized, (3) the initial consultation occurred, (4) the infant was admitted to the hospital, (5) chemotherapy was started and (6) transplant occurred. The timeline is shown in infant age or "days old" where day of birth = 0 days old. The goal of transplant occurring prior to thirty days is highlighted by gray shading beyond this time point.

Figure 2. Neurodevelopmental Outcomes Assessed 1-year after Transplant and Over Time Infants underwent developmental testing using the Bayley Scales of Infant Development, third edition (BSID-III) at 1-year post-transplant (Panels A-B) and at additional time points after transplant (Panels C-G). Composite scores were assigned for Motor, Language, and Cognitive development (Panel A). Composite scores are classified as "Extremely Low" (69 and below), "Borderline" (70–79), "Low Average" (80–89), "Average" (90–109), "High Average" (110–119), "Superior" (120–129), and "Very Superior" (130 and above). Subscales were also assigned for Gross Motor, Fine Motor, Receptive Language, Expressive Language, and Cognitive development (Panel B). Growth scores (mean: 500, SD: 100, range 200-800) were calculated using raw scores to reflect longitudinal growth independent of age[15] and is helpful in showing ongoing development in infants/children with low age-based scores. Growth scores are presented for individual patients (Patients 1-5) over time for the Subscales: Gross Motor (Panel C), Fine Motor (Panel D), Receptive Language (Panel E), Expressive Language (Panel F) and Cognitive (Panel G).

Figure 3. Nerve Conduction Studies (Panels A-E) and Brainstem Auditory Evoked Response (BAER) Results (Panel G) Over Time. Infants underwent nerve conduction studies before and after HCT. Motor neuropathy is present and progressive in most infants in the upper (Panels A and B) and lower extremities (Panel C and D). Similarly, sensory neuropathies are present and progressive (Panels E and F). BAER studies were abnormal in all patients tested over time (Panel G).

Table 1. Results of Newborn Screening, Confirmatory Testing and Pre-transplant Studies.

Study or Testing	Patient (normal values)					
	1	**2**	**3**	**4**	**5**	**6**
GALC enzyme activity assayed on DBS sample, in nmol/ml/hour	0.11 (>0.55)	0.1 (>0.4)	0.01 (>0.4)	0.03 (>0.55)	0.15 (>0.4)	10% (>13% of daily mean value)
GALC enzyme activity in leukocytes, in nmol/ml/mg protein	0.05 (>0.15)	0.03 (>0.15)	0.01 (>0.15)	0.34 (≥1.2)	0.09 (>0.15)	0.61 (>1.2)
Psychosine assayed on DBS sample, in nmol/L	24 (<2)	61 (<2)	56 (<3)	73 (<2)	38 (<3)	35 (<2)
Protein, CSF in mg/dL	117	386	348	332	444	446
Mutation analysis	c.379C>T (p.R127X); Del30kb	c.1884dupA (p.Trp629fs); del exon 8 (entire)-exon 9 (portion)	Del30kb; Del30kb	c.387C>G (p.Tyr129Ter)	c.1270C>T, (p.Gln424Ter); Del30kb	c.1723_1724insT (p.G575Vfs*10); c.1913G (p.G638V)
MRI	Abnormal	Normal	Abnormal	Normal	Normal	Abnormal
NCS	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Not Done
BAER	Abnormal	Abnormal	Abnormal	Abnormal	Abnormal	Not Done
VEP	Abnormal	Abnormal	Normal	Normal	Normal	Not Done
EEG*	ESTs	Normal	ESTs	ESTs	Normal	ESTs
Total number of abnormal studies	4	3	3	2	2	1

GALC, galactocerebrosidase; DBS, dried blood spot; PCR, polymerase chain reaction; MRI, magnetic resonance imaging; NCS, nerve conduction studies; BAER, brainstem auditory-evoked responses; VEP, visual evoked potentials; EEG, electroencephalogram; EST, excessive sharp transients; *ESTs were not considered abnormal for this purpose.

Table 2. Transplant Characteristics and Outcomes.

	Patient					
	1	**2**	**3**	**4**	**5**	**6**
HLA matching*	5 of 8	6 of 8	7 of 10	9 of 10	7 of 10	7 of 10
Total nucleated cell dose (x10^7/kg)	12.4	9.1	41.8	49.9	31.7	33.1
Days to neutrophil engraftment+	24	15	13	11	12	14
Days to platelet engraftment+	54	63	26	42	17	49
First post-HCT Chimerism (% Donor)±	98% WB 98% CD3 98% CD15	96% WB 97% CD3 96% CD15	98% WB 98% CD3 98% CD15	95%WB	98% WB 97% CD3 98% CD15	100% WB 100% CD3 100% CD15
Most recent HCT Chimerism (% Donor)±	>98% WB	>98% WB	92% WB 99% CD3 87% CD33	>95% WB	58% WB 57% CD3 43% CD33	83% WB
Psychosine (in nmol/L) at 1-year§	12	17	12	19	13.2	3.6
Acute GvHD, maximum grade (organs involved)	(possible mild upper gut)			2 (skin)	3 (skin, gut)	
Feeding intolerance post-HCT	Yes	Yes	Yes	Yes	Yes	Yes
Transplant complications¶		CMV viremia, Renal insufficiency, Pericardial effusion, AIHA	AIHA		Colonic perforation related to colonoscopy	VOD, Renal insufficiency, Bacterial infection

HLA, human leukocyte antigen; HCT, hematopoietic cell transplant; WB, whole blood; CD3, percentage of $CD3^+$ cells; CD15, percentage of $CD15^+$ cells; GvHD, graft versus host disease; CMV, cytomegalovirus; AIHA, Autoimmune Hemolytic Anemia; VOD, veno-occlusive disease; P, Patient *HLA loci considered in matching included A,B,C, DRβ1 if matching 8 loci and DQ if matching at 10 loci; +Defined as the first of 3 consecutive post-HCT days with an absolute neutrophil count (ANC) ≥500 cells/mm^3 or the first of 3 consecutive days with an untransfused platelet count ≥20,000 /mm^3; ±First post-engraftment restriction fragment length polymorphism performed around day +30 post-HCT. §Psychosine levels measured on dried blood spot (normal <2 nmol/L, P1, P2 and P5) or whole blood samples (normal <10 nmol/L; P3, P4, P6). ¶Complications were mild and self-resolved [renal insufficiency (P2 and P6)] or required treatment and resolved [CMV viremia (P2), pericardial effusion(P2), AIHA (P3), perforation (P5), VOD (P6), bacterial infection (P6)].

Figure 1

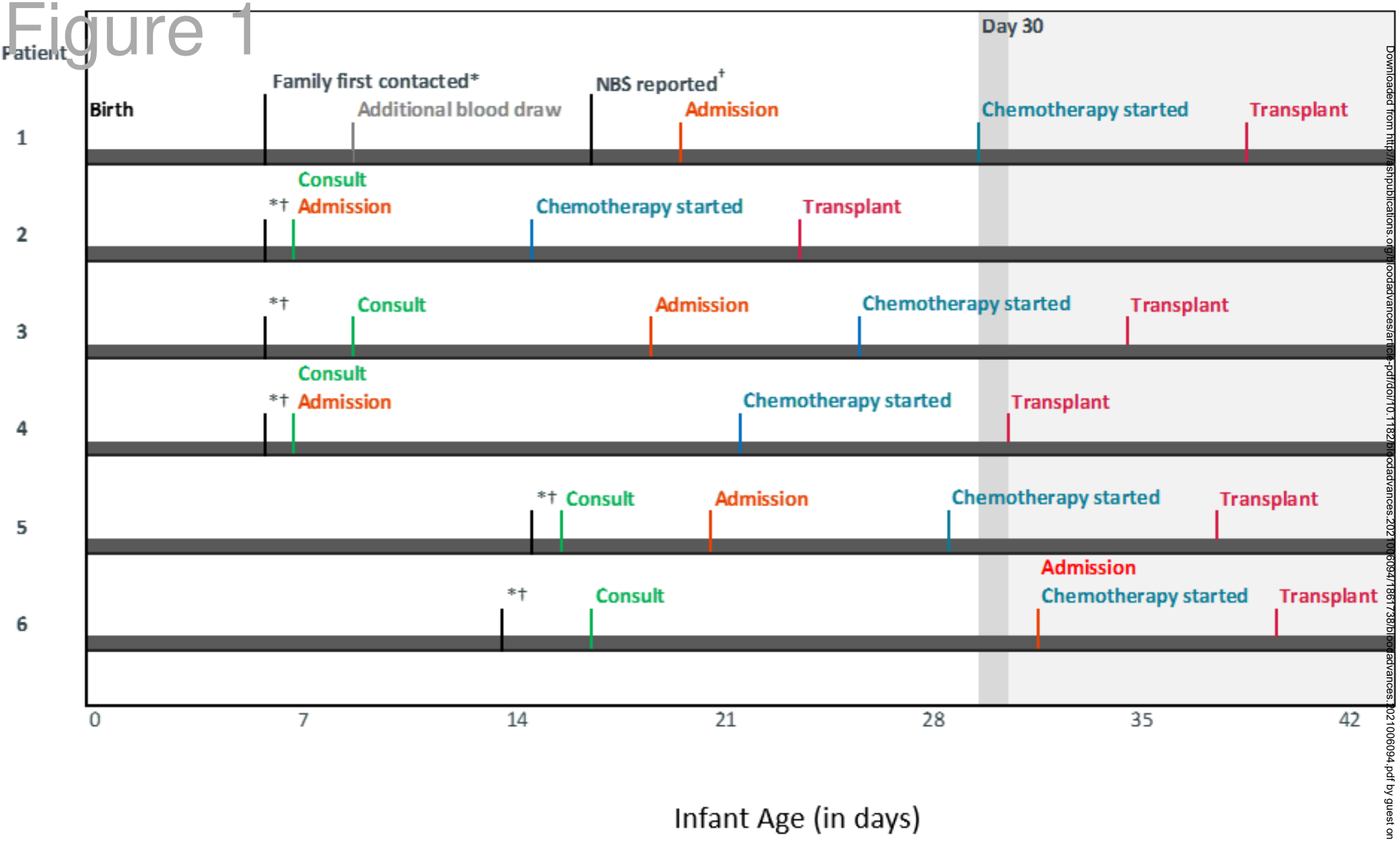

Figure 2

Figure 2. Neurodevelopmental Outcomes.

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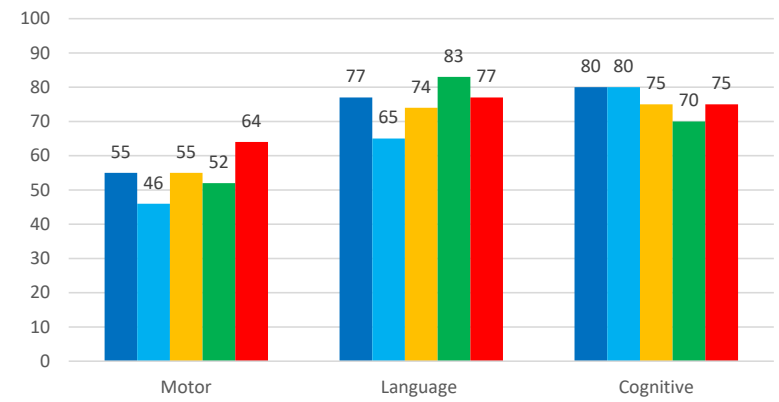

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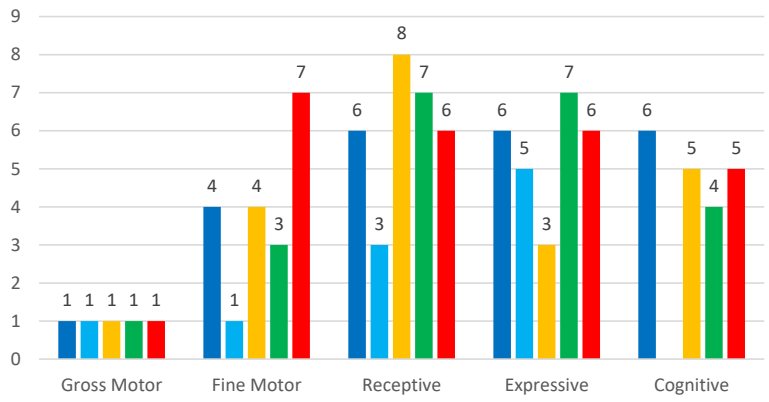

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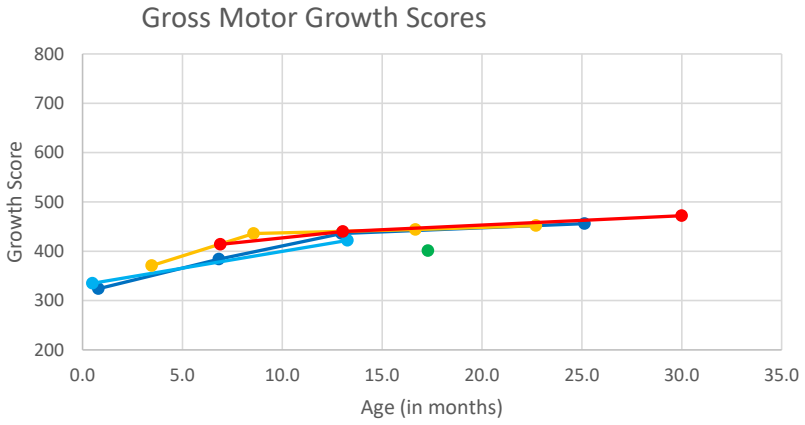

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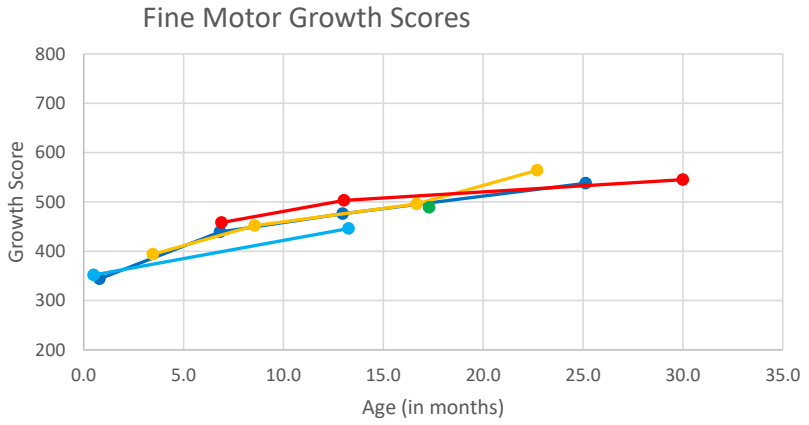

Figure 2

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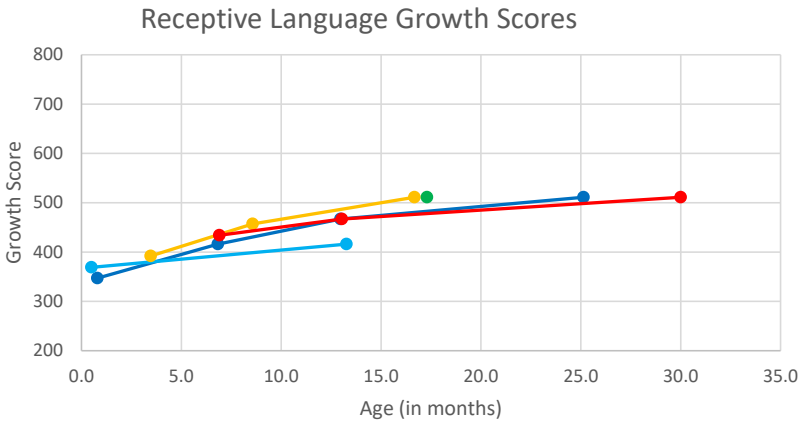

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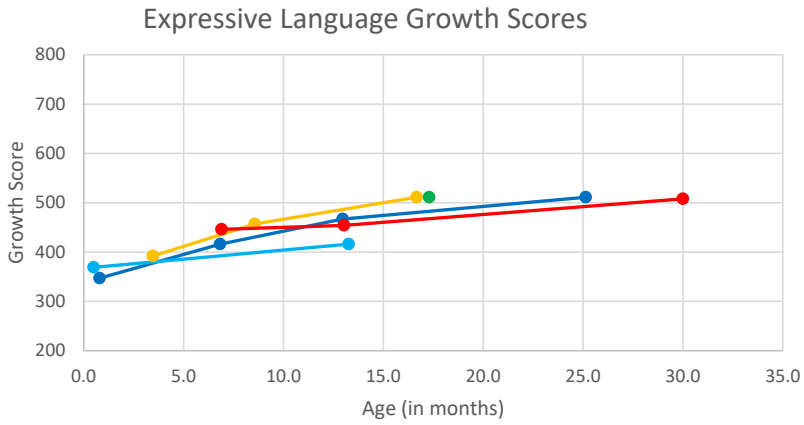

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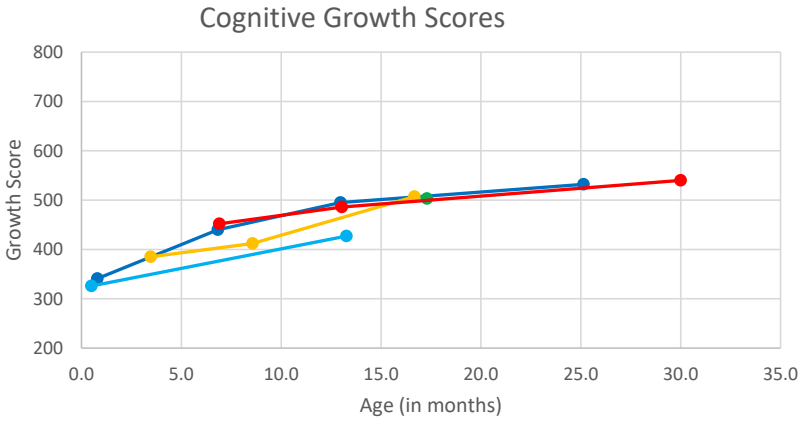


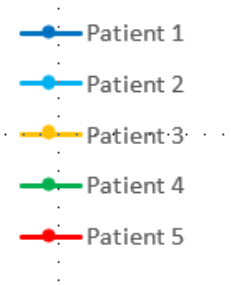
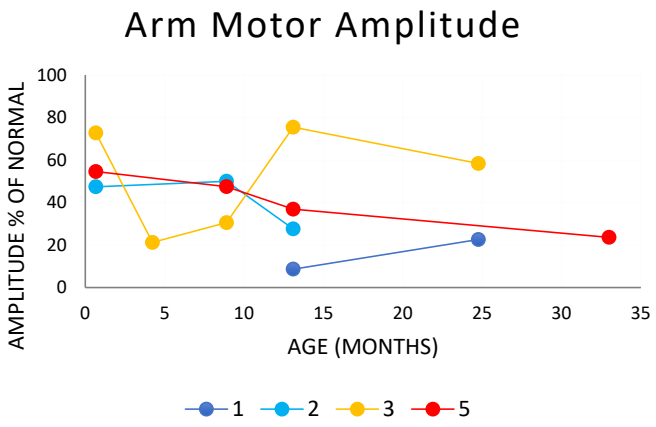


Figure 3

A



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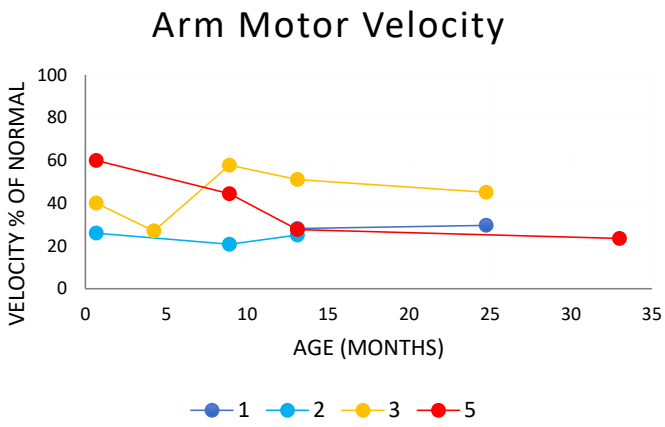

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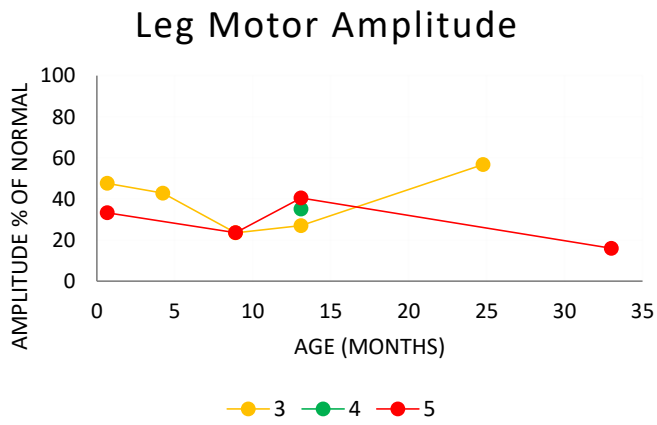

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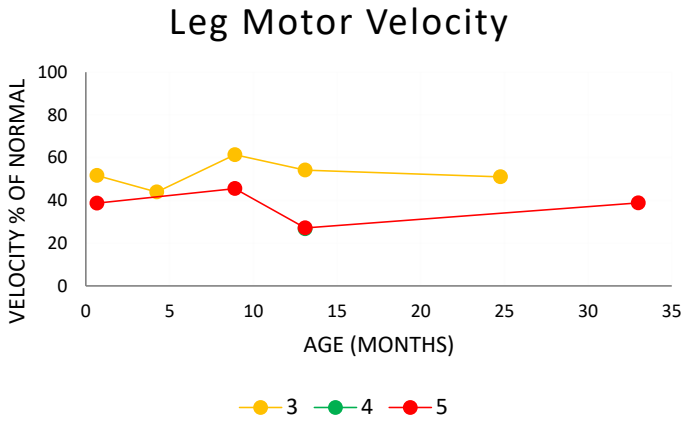

Figure 3

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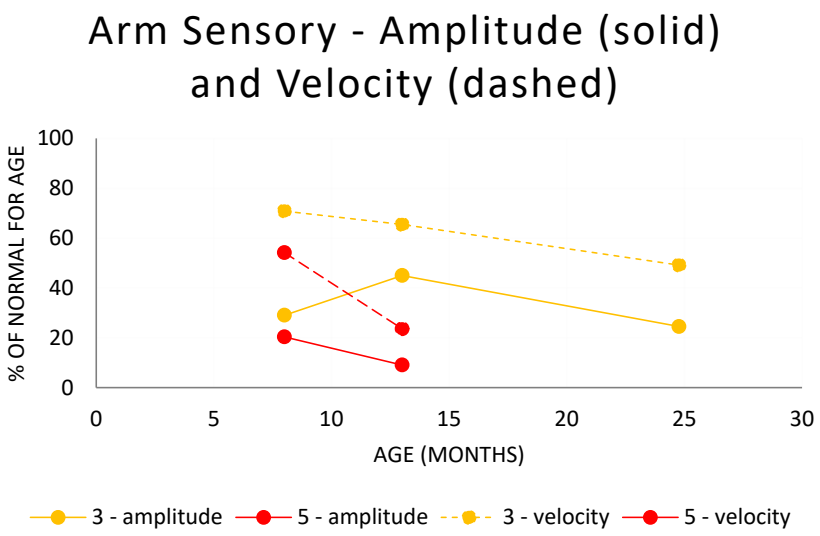

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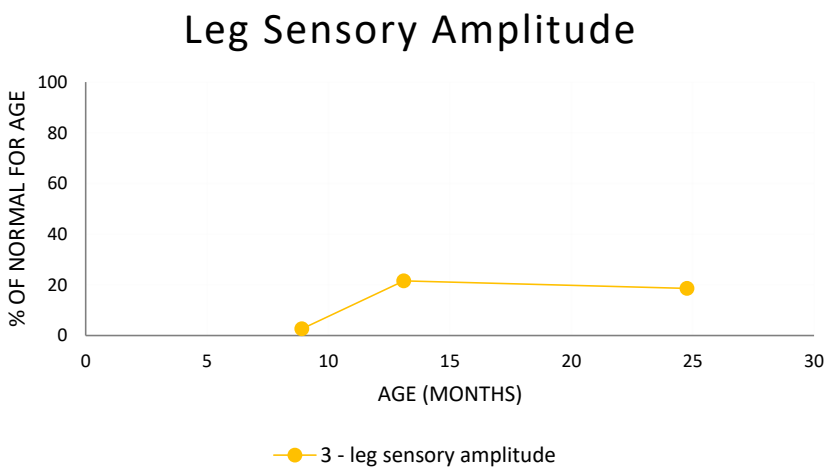

Figure 3

G