A Novel and Effective Process For Brain and Tissue Banking For Rare Diseases

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INTRODUCTION

Lysosomal storage disorders that affect the brain are rare (currently estimated at 1:2,000 to 1:7,000) and geographically dispersed.[1] Brain tissue is difficult to recover and often inaccessible due to the geographical location of the patient at the time of death and the limited time before the tissue decomposes. For the treating physician, it is a difficult topic to discuss and often there is no availability of a pathologist geographically close to where the child dies to coordinate the procedure within the short time frame necessary to access the brain for tissue retrieval.

Research in rare diseases is still seeking to elucidate the natural progression of the disease, identify biomarkers that predict disease burden, and translate basic research and disease models in animals to help understand the effects of a potential therapy.[2] The brain is one of the least understood organs in the human and extremely different in growth and development when compared to animal models. Our novel brain bank compares data from standardized neurodevelopmental evaluations of patients participating in a natural history study. This allows for direct comparison of the brain pathology with the child's functional, neurophysiological and neuroradiological testing. Analysis of human brain tissue offers the opportunity to better understand mechanisms of the disease process and how they relate to these measures. Additionally, the collection of blood and CSF provides another avenue to correlate findings and develop biomarkers of disease. Most biobanks lack this additional information critical to the translational model. We report on a brain and tissue banking model for rare diseases that has successfully procured specimens from patients across 12 US states and Mexico for the past 8 years.

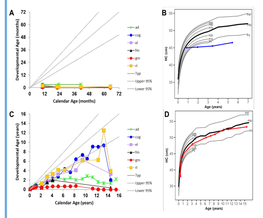

Figure 1. Neurocognitive development across multiple domains (A) without umbilical cord blood transplantation (UCBT) (case 1) and (C) after UCBT (case 2) using age-equivalent scores (i.e., developmental age). Head circumference (HC) development (B) without UCBT (case 1) and (D) after UCBT (case 2). The colored lines in panels (A,C) represent individual domains. The colored lines in panels (B,D) represent case 1 (blue) and case 2 (red). In all panels, the area between the gray lines represents typical development. ad, adaptive behavior; cog, cognitive; el, expressive language; fm, fine motor; gm, gross motor; rl, receptive language. P3, P5, P10, P25, P50, P75, P90, P95, and P97 represent percentiles (P).

METHODS

The biorepository was established in 2015 to house and study the brain and other tissues at the Program for the Study of Neurodevelopment in Rare Disorders (NDRD) at the University of Pittsburgh. A team consisting of a parent coordinator experienced with the process, a neurodevelopmental pediatrician, a neuropathologist, a technician, and a statistician was formed to obtain brain donations. The parent coordinator works with families/caregivers, local physicians, social workers, funeral homes and pathologists to schedule retrieval of the tissues via autopsy. The clinician brings up the topic to the family at an early stage before the time of acute deterioration is approaching and provides information about the parent advocate so they can reach out to them if interested and they are encouraged to do so before the time of crisis. The pathologist, designs the extraction protocol and communicates directly with the pathologist extracting the brain. The statistician keeps records of the data and help with the analysis. The team is also part of a committee that reviews requests for the use of sample-based, hypothesis driven applications that are likely to advance therapy for rare disease patients.

RESULTS

Age range at death	Sex	Disease Subtype	Underwent transplantation
0-12 months	M	Late infantile Krabbe disease	No
36 months +	F	Early infantile Krabbe disease	Yes
0-12 months	F	Late infantile Krabbe disease	No
36 months +	F	Sanfilippo Type A	Yes
12-36 months	F	Early infantile Krabbe disease	No
0-12 months	M	Late infantile Krabbe disease	No
36 months +	F	Sanfilippo Type A	No
12-36 months	M	Early infantile Krabbe disease	No
12-36 months	M	Early infantile Krabbe disease	No
12-36 months	M	Late infantile Krabbe disease	No
12-36 months	M	Early infantile Krabbe disease	No
12-36 months	M	Early infantile Krabbe disease	No
36 months +	M	Late infantile Krabbe disease	No
36 months +	M	Early infantile Krabbe disease	No
0-12 months	M	Early infantile Krabbe disease	No
36 months +	M	Gaucher disease	Yes
36 months +	M	Early infantile Krabbe disease	No
12-36 months	F	Early infantile Krabbe disease	No
12-36 months	M	Early infantile Krabbe disease	No
12-36 months	F	Early infantile Krabbe disease	No

Table 1. Current inventory of samples stored in the biorepository. Key information about each sample is tracked including, age at death, sex, disease subtype, and transplantation status.

To date, 21 donations have been made to the biorepository; 18 Krabbe cases, 2 Sanfilippo, one Gaucher type 2. Of the Krabbe donations, one patient was transplanted as a newborn, other cases are of various stages of disease progression. Current biorepository inventory is reported in **Table 1**.

Three peer-reviewed manuscripts have been published using the samples from the biorepository and other projects are being studied for publication. Most recently, Kofler *et al* used brain and tissue samples from the biorepository to report on neuropathology differences observed in 2 infantile Krabbe patients[3]; differences in neurodevelopmental outcomes (**Figure 1**), neuroradiological findings (**Figure 2, Figure 3**), and neuropathology (**Figure 4**) are attributed to differences in treatment: one patient underwent umbilical cord blood transplantation at 4 weeks of age and the other patient did not undergo transplant (ie. disease progressed in accordance with published natural history).

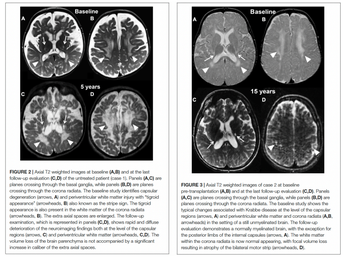

FIGURE 2 | Axial T2-weighted images at baseline (**A,B**) and at the last follow-up evaluation (**C,D**) of the untreated patient (case 1). Panels (**A,C**) are planes crossing through the basal ganglia, while panels (**B,D**) are planes crossing through the corona radiata. The baseline study identifies cerebellar degeneration (arrows, **A**) and periventricular white matter injury with "typical appearance" (arrowheads, **B**; also known as the stripe sign. The typical appearance is also present in the white matter of the corona radiata (arrowheads, **B**). The extra-axial spaces are enlarged. The follow-up examination, which is represented in panels (**C,D**), shows rapid and diffuse deterioration of the neuroimaging findings both at the level of the cerebellar regions (arrows, **C**) and periventricular white matter (arrowheads, **C,D**). The volume loss of the brain parenchyma is not accompanied by a significant increase in caliber of the extra-axial spaces.

FIGURE 3 | Axial T2-weighted images of case 2 at baseline pre-transplantation (**A,B**) and at the last follow-up evaluation (**C,D**). Panels (**A,C**) are planes crossing through the basal ganglia, while panels (**B,D**) are planes crossing through the corona radiata. The baseline study shows the typical changes associated with Krabbe disease at the level of the capsular regions (arrows, **A**) and periventricular white matter and corona radiata (**A,B**, arrowheads) in the setting of a still unmyelinated brain. The follow-up evaluation demonstrates a normally myelinated brain, with the exception for the posterior limbs of the internal capsules (arrows, **A**). The white matter within the corona radiata is now normal appearing, with focal volume loss resulting in atrophy of the bilateral motor strip (arrowheads, **B**).

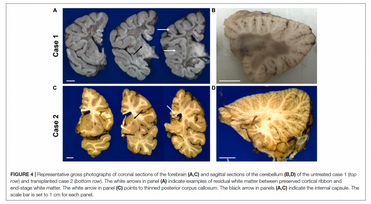

FIGURE 4 | Representative gross photographs of coronal sections of the forebrain (**A,C**) and sagittal sections of the cerebellum (**B,D**) of the untreated case 1 (top row) and transplanted case 2 (bottom row). The white arrows in panel (**A**) indicate examples of residual white matter between preserved cortical ribbon and end-stage white matter. The white arrow in panel (**C**) points to thinned posterior corpus callosum. The black arrow in panels (**A,C**) indicate the internal capsule. The scale bar is set to 1 cm for each panel.

CONCLUSIONS

Collecting tissues for rare disease research presents multiple challenges, including availability of samples, geographic location of the patient at the time of death, and ability to correlate observations with clinical findings. Our novel brain and tissue banking model allows for efficient collection and storage of samples but also enables comparison to disease-specific, prospectively designed natural history studies. This is beneficial in furthering translational research in rare diseases and is crucial to understanding mechanisms that are specific to humans and that provide valuable insights to the scientists working on the therapeutic development for the disease. Finally, our biorepository models allows families to provide meaning of their child's passing and attribute value to their loved one's life.

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ACKNOWLEDGEMENTS

We are grateful to the families who generously donated their loved ones' tissues.

NDRD staff & Biorepository staff

PARTNERS FOR KRABBE RESEARCH