Pediatric Demyelinating Disease Microbiome RFA

Frequently Asked Questions (FAQ)

1. **Why was the pediatric MS microbiome RFA created?**

The present RFA was launched to permit microbiome studies to be added to research already supporting a longitudinal, prospective cohort of children with CNS acquired demyelinating syndromes (ADS) from whom detailed new and archived clinical, genetic, immunological, and research-quality MRI data which have been and will be obtained.

2. **What is the core purpose of the present microbiome RFA?**

The core purpose is to explore the gut microbiome in the context of a comprehensively characterized pediatric ADS cohort. The microbiome RFA was proposed and funded following the successful review and granting of support (July 1, 2015-June 30, 2018) to the current Canadian Pediatric Demyelinating Disease study (CPDDS). Microbiome research was beyond the scope and expertise of the CPDDS project and investigators and was not included in the recently funded initiative. However, the potential importance of the gut microbiome in shaping MS risk and/or disease activity has been recently emphasized, and the opportunity to explore such studies in a large pediatric cohort (including newly enrolled patients-see below) was felt to be worthy of additional support.

3. **What are the Aims of the CPDDS study?**

Our **objectives** are (i) to evaluate the progressive and long-term aspects of MS disease burden in pediatric MS in terms of health-related quality of life (HRQOL) and cognitive function; (ii) to determine the contribution of potentially modifiable factors (physical activity, depression and anxiety) to HRQoL; (iii) to determine the impact of pediatric-onset MS on the health service utilization of patients and their parents; (iv) to evaluate MRI metrics of whole brain and cortical tissue injury and their relationship to our defined clinical outcomes; and (v) to pursue novel immunological studies of accelerated immune aging and dysregulation of immune cell subset balance and the extent to which these correlate with the relapsing and non-relapsing aspects of pediatric-onset MS. Collectively these objectives will robustly capture a multifaceted view of the progressive impact of pediatric MS from childhood through early adulthood on affected individuals.

Aim 1: To assess the extent and progressive burden of pediatric-onset MS on patient-centered outcomes and on patient and parental health services utilization.

Aim 2: To define the extent and progressive burden of pediatric-onset MS using global and regional brain MRI volumetric and tissue integrity measures and to assess the relationship of these MRI metrics with patient-centered outcomes and immunological features.

Aim 3: To define peripheral immune abnormalities of individual children with MS, investigate mechanisms underlying development of such abnormalities, and examine how persistence/evolution of these immune abnormalities over time relates to the evolution of focal, regional and global brain imaging metrics of tissue integrity and ultimately of clinical measures of the cumulative disease burden of pediatric MS.

4. **What are the resources already in place as part of the Canadian Pediatric Demyelinating Disease Study (CPDDS)?**

The CPDDS includes 23 sites (22 in Canada and The Children's Hospital of Philadelphia, see **Table 1**). Each site has a dedicated site PI and a site coordinator. Specific resources (which are fully supported) include:

- Site Coordinators (stipend model of support)
- CPDDS Research Management team (housed at the Research Institute, Hospital for Sick Children)- highly experienced at contract, IRB, sample organization, data entry and organization
- Standardized case report forms (which can be modified to include additional questions as per microbiome applications)
- Web-enabled database (Medidata Rave, housed at the Advanced Health Research Center, University of Toronto)
- Centralized biorepository (Tissue Metrix format, housed at the Experimental Therapeutics Lab, Montreal Neurological Institute)
- Centralized MRI analysis center (housed at the McConnell Imaging Center, Montreal Neurological Institute
- Standardized Operating Procedures (SOPs) for serum, DNA, peripheral blood mononuclear cell, and CSF samples that have been carefully validated
- Shipping contracts for all sites

5. **What is the protocol for the CPDDS?**

Table 2 lists all of the tests and procedures proposed for the CPDDS participants. The CPDDS current study has "established" participants (children with monophasic ADS and children identified with MS enrolled from 2004-2015, all of whom will be re-consented for the current CPDDS study. Please note that consents signed between 2004-2015 specifically permit use of archived data and samples for research), incident MS participants (to be enrolled within 6 months of first MS attack; or at the time of incident attack of they meet 2010 McDonald criteria for MS diagnosis at that time point), and age-, sex- matched healthy controls (matched to the established and incident MS groups).

- Established MS (n=80-90)
- Established monophasic ADS (n=370-390)
- Healthy controls (n=200) cross-sectional sample

- Incident MS (n=40, to be enrolled at CHOP and SickKids only)
- Healthy controls (n=120, to be enrolled and matched to MS groups)

Full protocol documents, the final study manual, and all of the SOPs for the study are under final review and will be submitted for ethics approval at all participating sites in July. At present, the protocols indicate that stool collection (precise sample specifications to be provided by the successful microbiome team) will be obtained (as listed in **Table 2**) from all established participants, from new incident MS patients, and from the healthy controls. We have indicated in the ethics documents that an early life and diet questionnaire will be employed (anticipating that this will be one of the components of the microbiome proposal), and have

used our current "DSunQ" questionnaire (attached) as a place-holder. Standardized questionnaires (such as the PedsQL) are available on-line and are not attached here.

Case report forms/database capture date of incident ADS, age at all time points, sex, presenting features (at onset and at relapses for the MS group), details of clinically obtained CSF (cell counts, oligoclonal bands), serum NMO status (clinically tested, and tested as part of our research studies for the first 302 participants), gestational age at birth, complications in pregnancy, breastfed (yes/no, how many months), vaccination history, vaccination exposure within 30 days of incident event, other medical illnesses, all medications (dates as well), and family history of MS or other autoimmune disease (in addition to asking parents at time of enrolment, a formal genetic interview was performed for over 270 participants and is being completed for the remaining 130 to date).

6. What is the timeline for participant engagement in the CPDDS?

All required documents for study launch across the participating sites will be finalized by July 30, 2015. Study approval at each site is anticipated no later than Oct 30, 2015, but is anticipated by Sept 1, 2015 at CHOP and SickKids.

A study launch meeting will be held Sept 20-22, 2015. All CPDDS PIs, all investigators (including the successful microbiome application team), the study steering committee, and all site PIs and coordinators are invited to attend in person.

Contact with established participant groups by Site Coordinators will commence immediately upon ethics approval (start date anticipated in September for SickKids and CHOP, and across all sites by December). Enrolment of incident MS patients and new healthy control participants will commence in September (at the two enrolling sites, SickKids and CHOP).

7. What has been acquired through the CPDDS to date?

The CPDDS began July 1, 2004. To date, we have enrolled over 450 children with incident CNS demyelination (hereafter referred to as having acquired demyelinating syndromes, ADS). All participants were enrolled within 30 days of incident ADS, and have been prospectively evaluated at baseline, 3, 6, 12 months and annually. While the vast majority of study visits occurred in person, participants with monophasic ADS (defined by the absence of both clinical new attacks and by MRI studies demonstrating no new lesions) were offered telephone interview follow-ups over the last 2 years (especially those participants enrolled in the first few years in whom > 5 years of consistent absence of new disease activity was documented). The telephone interviews were detailed and included the patient-reported EDSS.

To date, our study has acquired:

- Prospective clinical data on over 450 children age <15 years 11 months at incident ADS
- Serum samples (>27,000 stored aliquots) generally obtained at all study time points
- DNA samples (>10,000 aliquots)
- CSF samples (when obtained for clinical purposes), approximately 650 aliquots
- Peripheral blood mononuclear cell samples (>1200)
- Research quality MRI (1.5T) scans (>2200)
- Quality of life questionnaires (since 2010)

- DSunQ questionnaire (completed by approximately 270 participants and families)
- 4 hour cognitive battery (SickKids, CHOP, and Calgary participants only, 2010-2014, at 6 and 24 months post-ADS: data being analyzed now, limited number of participants)

Table 3 summarizes the samples obtained per subject to date.

NOTE: Stool samples have not been obtained from the established CPDDS cohort.

8. **What potential resources are not funded as part of the CPDDS, and would be required as budget items for the microbiome applicants?**

- Costs for stool sample procurement
- Costs for stool sample processing (eg solid and liquid phase)
- Shipping costs for stool samples
- Costs of storing stool samples (either in Montreal or at the applicant site)
- Costs for modifying the CPDDS database for questions added by the microbiome application and for insertion of any new questionnaires (such as new diet questionnaire)
- Costs for centralized data entry of new questionnaires
- Costs of assays to generate microbiome data
- Data analysis costs related to the microbiome (or a partnered analytical plan with the CPDDS)
- Any costs related to study populations other than the CPDDS (if applicable)

NOTE: The CPDDS database is web-enabled, and site-direct data entry is completed at SickKids and CHOP. The data entry for all other sites is performed centrally at SickKids by trained data entry staff.

9. **Can applicants propose partnerships with other patient groups, resources, or teams?**

Microbiome applications can either be designed to work solely with the participant population of the CPDDS, or can partner the CPDDS with other established or emerging groups working on the microbiome in MS orother diseases. Partnered application models must clearly articulate how such partnered populations can be compared for already established data and/or how new information to be collected can be comparably obtained across all of the participants. Budget must be sufficient for such a partnership. Leveraged resource utilization is encouraged and should be clearly justified.

10. **Who will adjudicate the microbiome applications?**

A scientific advisory committee has been appointed to review the microbiome applications. The committee includes experts in MS and experts in microbiome research, as well as statistical advisors.

11. **What are the key metrics that will be adjudicated when reviewing the microbiome applications?**

Relevance to MS
Applicants will be required to outline what they feel will be the key findings of the work and how this is important to MS, and what their findings might lead to in terms of further research. In addition, the

applicants must provide a short (200 word) lay summary that emphasizes how their project is important to persons with living with MS.

Scientific Research Proposal

- **Scientific Quality:** The successful microbiome application will define scientific aims and hypotheses and defend the rationale for these with available literature. Evidence of peer-reviewed work in the sphere of microbiome research by one or more of the listed investigators will be viewed favorably.
- **Novelty:** Applicants should discuss how their proposed study will yield new information in the field and should defend why using a pediatric population is novel and informative.
- **Integration and Partnership**: Applicants should clearly delineate how the microbiome project will integrate and maximally utilize the already funded CPDDS components. A section describing how the PIs of the microbiome project will work with the PI team of the CPDDS should be described- although the details can be finalized at the September launch meeting described above. Please note that very important details of data access, publication and authorship collaborative agreements, sample access and usage between the microbiome applicants and the CPDDS, and oversight policies including an open-access future model should be briefly articulated, with a view to a detailed discussion between the successful microbiome team and the CPDDS. A collaborative partnership is envisioned and ultimately, creation of a shared resource for future studies should be considered a key objective.
- **Feasibility:** The methods section of the proposal should articulate clearly how sample procurement can be maximized including comments on how to motivate or facilitate patient/parentally-facilitated endorsement. Detailed SOPs are expected for sample handling and analysis. Questionnaires regarding early life, diet, and other exposures should be validated (if possible), or preliminary data from pilot or partnered projects should be provided. Particular attention should be paid to length of questionnaires and to challenges of anamnestic accuracy.
- **Analyses:** The analysis plan should broadly show how the hypotheses described in the aims will be tested. Applicants must show adequate power to test the hypotheses. Specifically, applications will be evaluated as to whether applicants enumerate and define the outcome variables and exposure variables clearly. Applicants should consider and describe covariates - and why these were chosen. The statistical model/approach selected must be clearly defined. Applications must specifically address the issue of multiple comparisons, and the sample size/power calculation should be provided and clearly tied to the analysis model chosen.

Budget

The budget must justify costs that relate to the microbiome, as well as briefly state where the applicants have used partnered resources. Please note that the Multiple Sclerosis Scientific Research Foundation is a non-profit organization, and does NOT pay overhead costs (indirect costs) nor PI salary support. In general, salary support for trainees should be for a period of 1-2 years, with the expectation that such trainees would be suitable candidates for doctoral or post-doctoral fellowship programs (and thus might need only one year of grant funded full salary, with other years supported by fellowships and grant-supported salary top-up). Refer to MSSRF Team Grant Terms, Condition and Policies for more information on allowable expenditure categories.

Table 1: CPDDS Sites

Site Number	Site ID	CITY	Institution
001	WIN	Winnipeg, MN	Winnipeg Children's Hospital
002	HAM	Hamilton, ON	McMaster Children's Hospital
003	RVC	Toronto, ON	Rouge Valley Health System Centenary Site
004	SJR	Saint John, NB	Atlantic Health Sciences Corporation
005	VIC	Victoria, BC	Victoria General Hospital
006	SAS	Saskatoon, SK	Royal University Hospital
007	LON	London, ON	London Health Sciences Centre Children's Hospital
008	VAN	Vancouver, BC	BC Children's Hospital University of British Columbia
009	CAL	Calgary, AB	Alberta Children's Hospital
010	OTT	Ottawa, ON	Children's Hospital of Eastern Ontario
011	HSC	Toronto, ON	Hospital for Sick Children
012	EDM	Edmonton, AB	Stollery Children's Hospital
013	SHE	Sherbrooke, QC	Université de Sherbrooke
014	CLM	Longueuil, QC	Hôpital Charles-LeMoyne
015	TRI	Mississauga, ON	Trillium Health Centre
016	KIN	Kingston, ON	Kingston General Hospital

017	SUD	Sudbury, ON	Sudbury Regional
018	MON	Montreal, QC	Montreal Children's Hospital Montreal Neurological Institute
019	IWK	Halifax, NS	IWK Health Centre, Halifax
020	WND	Windsor, ON	Hôtel-Dieu Grace Healthcare
021	CHP	Philadelphia, PA	Children’s Hospital of Philadelphia
022	STJ	St. John’s, NL	Janeway Children’s Health and Rehabilitation Centre
023	JUS	Montreal, QC	Centre hospitalier universitaire Sainte-Justine

Table 2: CPDDS Study Protocol (2015-2018)

	Incident MS (n=40)						**Established MS** (n=81)				**Mono ADS** (n=315)			**Healthy Controls** (n=120)
Visits	**V1 Baseline**	**V2 3 mths**	**V3 6 mths**	**V4 12 mths**	**V5 24 mths**	**UV**	**V1** 2015-16	**V2** 2016-17	**V3** 2017-18	**UV**	**V1** 2015-16	**V2** 2016-17	**V3** 2017-18	**V1**
Clinical & EDSS examination	✓*	✓*	✓*	✓*	✓*	✓	✓*	✓*	✓*	✓				✓ **Excludes EDSS**
Telephone interview											✓	✓	✓	
Barratt Social Status Scale	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓
HRQoL*	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓
Neurocognitive	✓		✓	✓	✓		✓	✓	✓					✓
Physical activity	✓	✓	✓	✓	✓		✓	✓	✓		✓ **GLTEQ only**	✓ **GLTEQ only**	✓ **GLTEQ only**	✓
3T MRI scans	✓	✓	✓	✓	✓		✓	✓	✓					✓
7T MRI scans (CHOP patients only)	✓	✓	✓	✓	✓									
Biological samples	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓				✓
Stool Samples (and questionnaire)	✓	✓	✓	✓	✓		✓	✓	✓		✓	✓	✓	✓
Mouth Swabs	✓[1]	✓	✓	✓	✓		✓	✓	✓					✓
Dermaspec (CHOP and SickKIds) and Skin tone	✓	✓	✓	✓	✓		✓	✓	✓					✓

HRQoL= PedsQL (patient and family modules), as well as questionnaires for depression and anxiety
GLTEQ = Godin Leisure Time Exercise Questionnaire

Table 3: samples obtained from the CPDDS 2004-2015

	TOTAL # SUBJECTS	# SUBJECTS WITH DNA	# SUBJECTS WITH SERUM	# SUBJECTS WITH PBMC	# SUBJECTS WITH CSF
PATIENT	408	403	404	177	92*
HEALTHY CONTROLS	71	65	70	62	1
TOTAL	**479**	**468**	**474**	**239**	**93**

CSF was obtained at the time of incident demyelination as part of clinical evaluation. Of the 92 samples available, 18 are from patients with confirmed MS.