

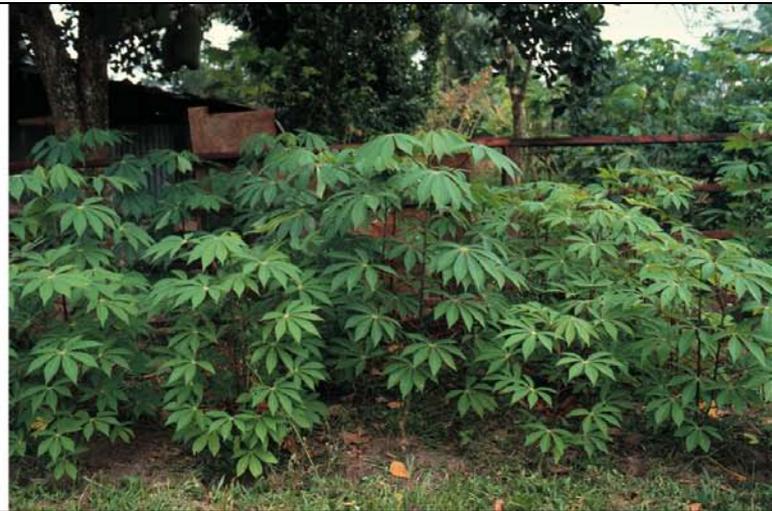
Food (cassava) Toxicity, Nutritional Deficiencies, and Genetics Lessons from R21-R01 studies

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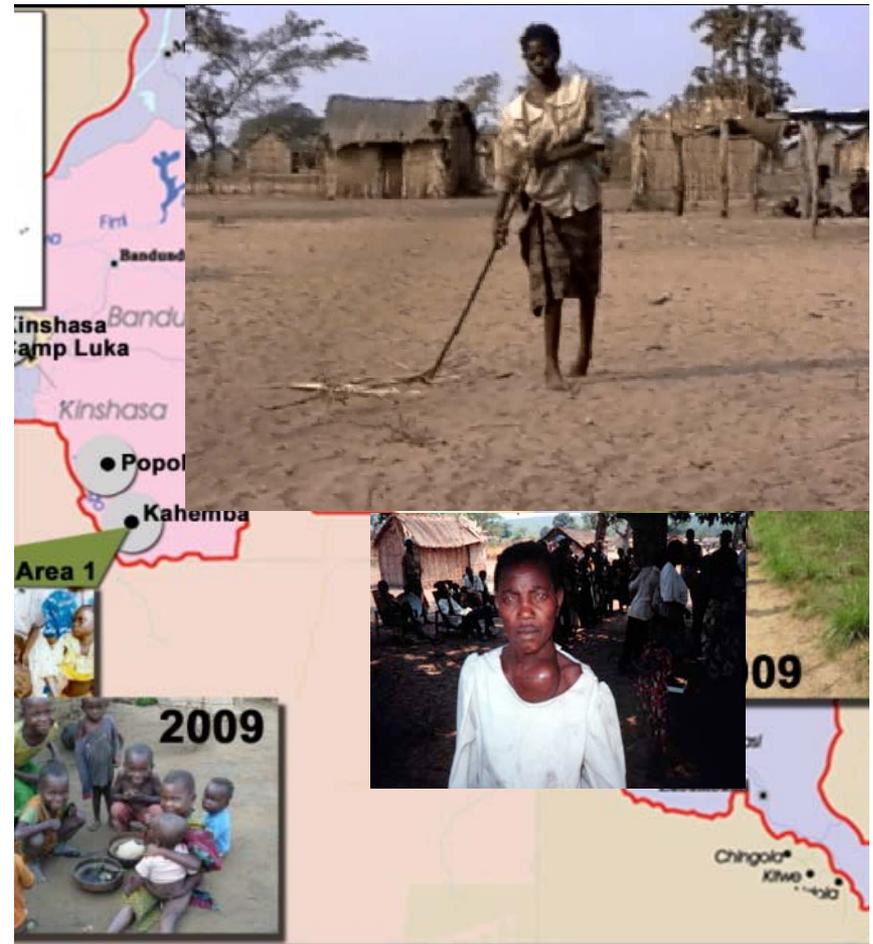
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Toxiconutritional Motor Neuron Diseases: Konzo vs. Lathyrism



R21-Phase

- Model development (clues to the markers and pathogenesis: oxidative damage, Kassa et al., 2011)
- Capacity building
 - Neuroscience Research Design and Methodology
 - Neuroepidemiology
 - NeurotoxicityMajor partners: OHSU/UHSU/Verona-Italy/DRC
- Research team building and R01 development
 - Hypothesis-driven work
 - Capacity building: neuropsychology and molecular biology
 - Major partners: OHSU/MSU/Bergen-Norway/DRC Gov. and Research Institutes



R01-Hypothesis and Major Aims



Hypothesis: risk for cassava-associated motor cognition deficits is modulated by nutritional toxicity, genetic (TST) polymorphisms, or their interactions

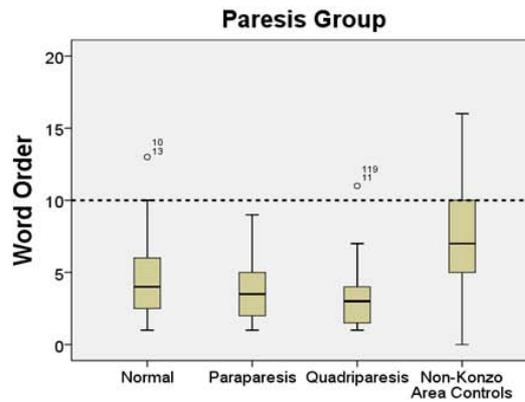
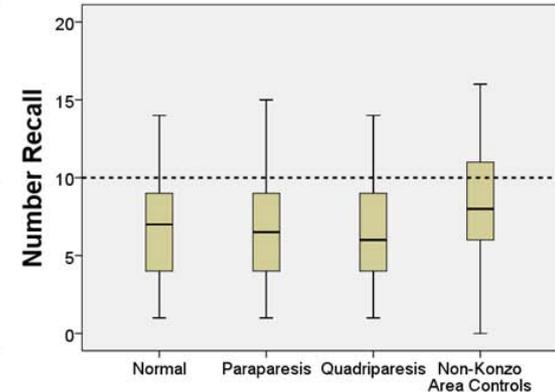
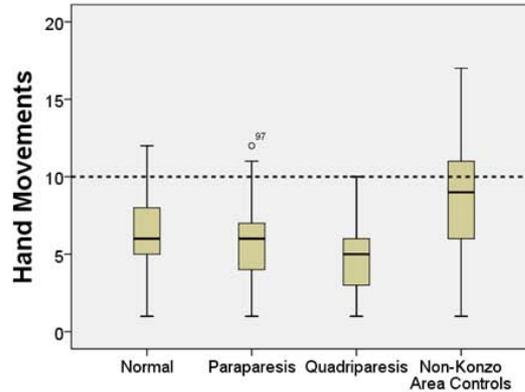
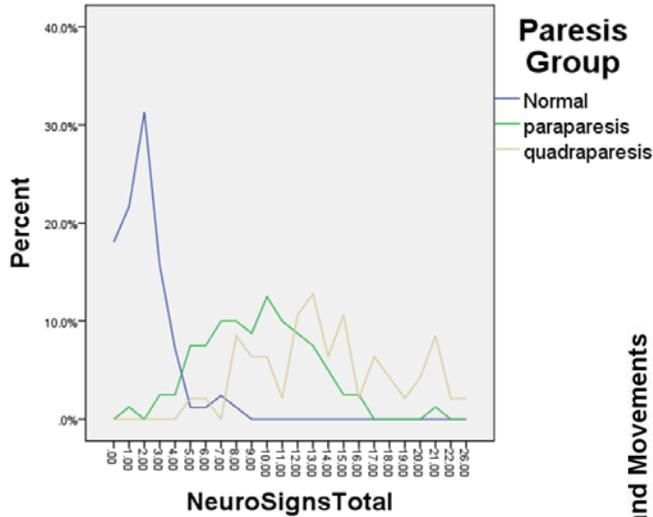
Specific Aims:

- Neuropsychological profiling of cassava-reliant children
- Genetic (TST) sequencing)
- Biomarker studies

Methods

- Neuroepidemiology
- Neuropsychology of cassava toxicity (BOT-2 and KABC-II)
- Biochemistry
- Enzymatic cyanide detoxification capabilities
- Tandem MS (proteomics) for carbamoylation studies
- LC/MS-MS for isoprostanes
- Genetic sequencing (Applied Biosystems Inc 3130XL Sequencer)

Critical Neuropsychology

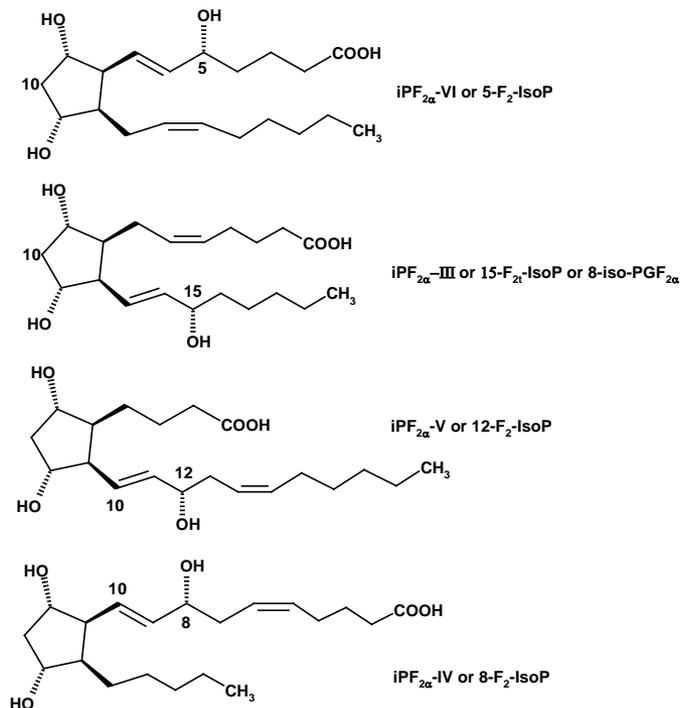


Exposure/Nutritional Markers

Severity/ Parameters (Units)	Mildly Affected	Severely Affected	Wilcoxon rank- sum <i>p</i> -values
Urinary Thiocyanate ($\mu\text{mol/l}$)	344 (172-688)	344 (17.2-1032)	0.71
Albumin ($\mu\text{mol/l}$)	602.63 (443.87- 687.60)	450.02 (261.62- 693.19)	0.00
Cyanide detoxification capabilities	146.90 (36.22-435.32)	140.24 (35.92-221.07)	0.15
Triglycerides (mmol/l)	0.98 (0.622-1.81)	1.27 (0.66-3.88)	0.01
Thyroid Stimulating Hormone (TSH, uIU/mL)	3.48 (0.99-9.27)	3.04 (1.26-7.04)	0.63
Free-T4 (Thyroxine, ng/dL)	1.1 (0.7-1.6)	1 (0.8-1.6)	0.35

Subclinical hypothyroidism is found. No difference in total proteins, however, differences suggested in terms of quality of proteins (PTM?), APOA and mu IG
(Proteomics In Progress)

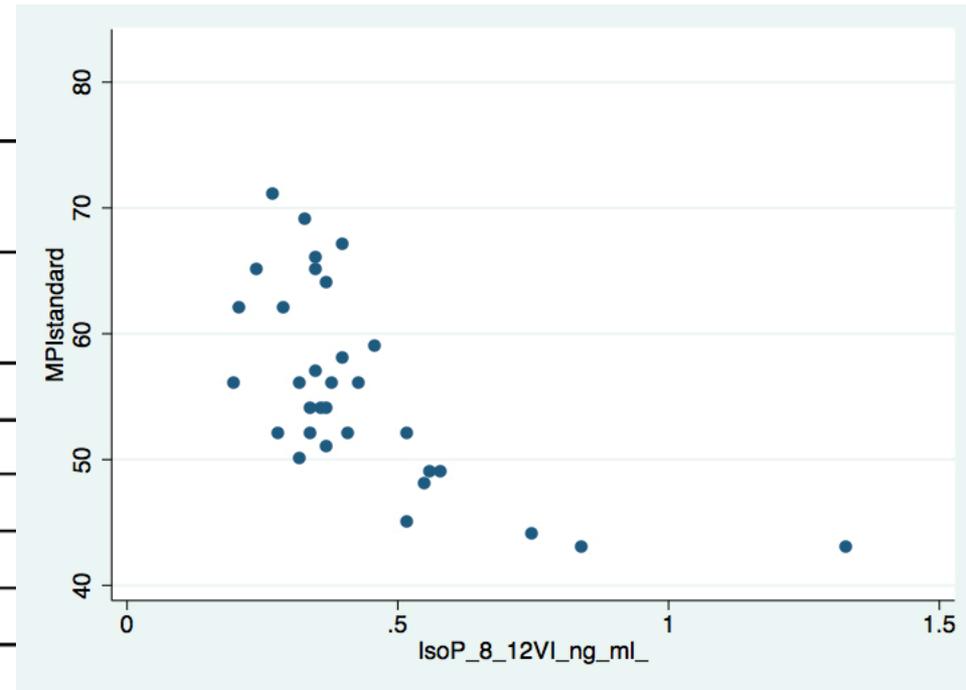
LC/Tandem MS Isoprostanes



Severity/ Parameters (Units)	Mildly Affected	Severely Affected	Wilcoxon rank-sum <i>p</i> values
<i>IsoP-III</i> (ng/ml)	0.24 (0.12-0.55)	0.285 (0.11-0.84)	0.87
<i>IsoP-III-tot</i> (ng/ml)	2.68 (0.75-8.25)	3.065 (0.95-7.34)	0.84
<i>IsoP-VI</i> (ng/ml)	0.25 (0.16-0.40)	0.285 (0.14-1.53)	0.35
<i>IsoP-VI-tot</i> (ng/ml)	2.94 (1.9-4.15)	2.98 (1.98-9.0)	0.38
<i>IsoP 8,12VI</i>(ng/ml)	0.35 (0.22-0.56)	0.445 (0.21-1.33)	0.02

Phenotypic Anchoring: MPI, Disease Severity, and Clinical Biochemistry

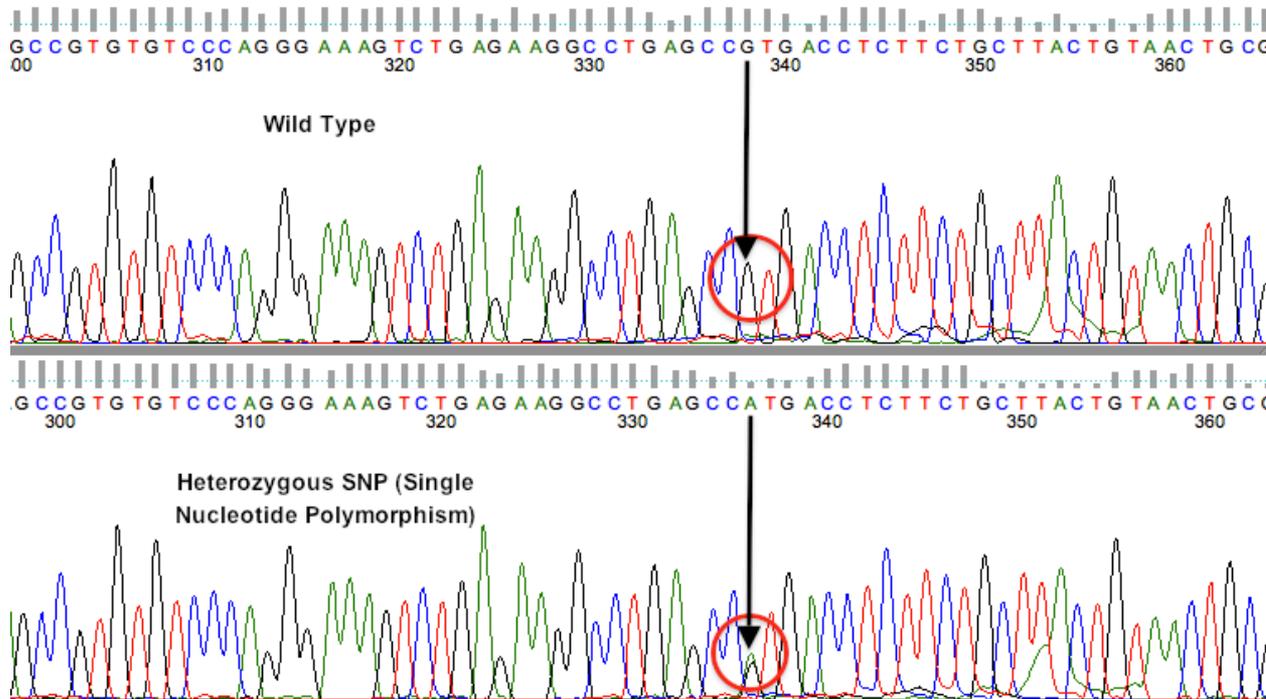
Biomarkers	Overall Spearman r	P-values		
<i>Motor Proficiency Total</i>	0.69	0.00		
<i>Albumin</i>	0.47	0.03		
<i>IsoP-III</i>	- 0.39	0.07		
<i>IsoP-III-tot</i>	- 0.43	0.05		
<i>IsoP-VI</i>	- 0.47	0.03		
<i>IsoP-VI-tot</i>	- 0.47	0.03		
<i>IsoP 8,12VI**</i>	- 0.57	0.01		
<i>Cyanide Detoxification Capabilities</i>	- 0.14	0.42	- 0.39	0.14



Phenotypic Anchoring: Motor Proficiency, Disease Severity, and Clinical Biochemistry

Biomarkers	Overall Spearman r	P-values	Spearman r severely affected	P-values
<i>MPI</i>	0.69	0.00	0.54	0.04
<i>Albumin</i>	0.61	0.00	0.33	0.26
<i>Triglycerides</i>	- 0.47	0.03	- 0.31	0.29
<i>IsoP-III</i>	- 0.14	0.52	- 0.63	0.02
<i>IsoP-III-tot</i>	- 0.22	0.33	- 0.63	0.02
<i>IsoP 8,12VI*</i>	- 0.47	0.03	- 0.43	0.13
<i>Cyanide Detoxification Capabilities</i>	0.18	0.28	- 0.24	0.39

Genetics-TST/MPST/APOE4: Evolution and Adaptation?



TST
G125>A
#A103>C
Glutamic > Aspartic acid
C43>T
G294>A*
G451>A*
Valine > Methionine
C169>T
Arginine > Cysteine
C345>T*
A184>G*
Methionine > Valine
A459>G*
Asparagine > Aspartic acid
MPST C350>T*
Proline > Proline
APOE-Exon4
T117>C
Valine > Alanine
C161>T
Alanine > Valine

TST Polymorphisms	Severe Disease	Mild Disease	Exact <i>p</i> -value
Yes	15	20	0.08
No	0	5	
MPST + TST Polymorphisms			
Yes	15	20	0.08
No	0	5	
APOE-exon 4			
Yes	0	3	0.28
No	15	22	

Future Research Directions

- Re-assess the global burden of cassava-associated neurodegeneration (Global Health Impact)
- Clinical/Community trials with novel antioxidant strategies
- **The Ethiopian dilemma: cassava into *lathyrus sativus* growing area, what next?**
- Genetic replication studies including **whole exome** (genome) sequencing for total genetic variation
- Metagenomic variations and risk for cassava-associated neurodegeneration (hypothesis-driven and search for probiotics)

FIC/NIH-Research Capacity Building

- Ethical IRB
- Enhance local laboratory capabilities for biochemical (molecular biology) and toxicological analyses
- Neuropsychology unit
- Cold chain and custody of QC work (DRC-Europe-USA)
- Small scale repository for specimens (blood, proteins, nucleic acids, urines, stools, and pathogens)
- Training and mentoring (7 PhD candidates from diverse background: MD, Public Health, Nutrition, Pharmacy, Biochemistry, and
- Sustainable IBRO funding (advanced school DRC and Neighboring countries)
- **Next: Complex Morbidity Unit/CRYOBANK for Complex, Neglected, and Rare Diseases (HTLV-III and IV?)**
- **Challenges: administrative, language, politics, OMICs cost**



R01-Acknowledgment

Oregon/OHSU and MSU

- **CROET & Neurology**

Morris Kim

Furaha Alex

Shawn Westaway

Mike Lasarev

- **Proteomics OHSU**

Larry David

John Klimek

Deb McMillen

- **OCTRI OHSU**

- **MSU**

Michael Boivin

International

- **University of Kinshasa and National Biomedical Research Institute, DRC**

Tamfum Muyembe

Kazadi Kayembe

Mumba Ngoyi

Nadege Ngombe

- **Ministry of Health, PRONANUT, DRC**

Banea Mayambu

- **Ministry of Higher Education, DRC**

Mashako Mamba

- **University of Nairobi**

Samuel Kimani

- **University of Bergen**

Thorkild Tylleskar