A Repeated Measures Metabolome−Wide Association Study of Malignant Glioma in U.S. Military Personnel

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INTRODUCTION

- **Malignant gliomas** represent approximately 80% of all primary malignant brain tumors, but their causes are still not fully understood
- Recent research suggests aberrant **metabolism may play a role in glioma tumorigenesis**, providing etiological clues
- **Military service members experience unique stressors and exposures** that may elevate brain cancer risk, and research to date focusing on military service members remains limited
- To investigate the role of metabolic patterns associated with malignant glioma, **we performed a Metabolic-Wide Association Study (MWAS) to identify metabolic biomarkers of glioma** using pre-diagnostic serum samples from a large, nested case-control study of 451 cases and 454 controls **[1]**

METHODS

Pre-dx Serum Samples 1988-2011

Department of Defense Serum Repository

Cases

DoD Cancer Registry (DCR)

- (ICD-O-3) codes 938-948

Defense Medical Surveillance System (DMSS)

- ICD-9 code 191 supported by relevant diagnostic Current Procedural Terminology (CPT) codes:
  - nervous system surgery: 61000-64999
  - cytopathology: 88104- 88199
  - cytogenetics: 88230-88299
  - surgical pathology: 88300-88399

Controls

- Matching criteria:
  - Sex
  - Year of birth
  - Race
  - # Serum samples
  - Date of serum sample

- **1-3 pre-diagnostic samples** from cases and individually matched controls
- Serum analyzed with **high-resolution untargeted metabolomics** via Dual-column liquid chromatography and electrospray ionization **(LC-MS)**
  - **Zero values** were considered < limit of detection (LOD)
  - Metabolic features were excluded if >20% zeroes in cases or controls
  - Zero values in retained features imputed with the **minimum observed value divided by √2**
- Differences in metabolic patterns were assessed using **linear mixed models**
  - controlling for sex, race, age, and branch of service
- **Metabolic pathway enrichment analysis** performed via mummichog algorithm, version 2.0 **[2]**
- Metabolic features **annotated** using MetaboAnalyst version 6.0 **[3]**
- Additional Analyses considered MWAS stratified by lag time of serum sample to diagnosis (**1-5 years; 5-20 years)**

RESULTS

Table 1. Summary of Study Population

		All Samples		1-5 Years Pre-Diagnosis		5-20 Years Pre-Diagnosis	
		Cases	Controls	Cases	Controls	Cases	Controls
N							
	Subjects	451	454	410	420	321	316
	Samples	1098	1085	550	554	481	469
Age		32(8)	32(8)	30(8)	30(8)	33(7)	33(7)
Sex							
	Male	88.7%	88.8%	87.8%	88.3%	89.2%	89.1%
	Female	11.3%	11.2%	12.2%	11.7%	10.8%	10.9%
Race							
	White	80.7%	81.0%	80.0%	80.3%	80.9%	81.4%
	Black	11.7%	11.3%	12.0%	11.4%	12.1%	11.7%
	Other	3.6%	3.6%	4.0%	3.8%	3.3%	3.4%
	Missing	3.9%	4.1%	4.0%	4.5%	3.7%	3.4%
Service							
	Army	35.7%	35.7%	34.5%	35.6%	37.0%	34.3%
	Marine Corps	12.7%	16.4%	15.1%	17.3%	10.6%	15.8%
	Navy	27.7%	35.3%	26.5%	31.9%	28.1%	39.0%
	Air Force	21.8%	12.5%	22.2%	15.2%	21.6%	10.7%
	Coast Guard	2.2%	0.1%	1.6%	0.0%	2.7%	0.2%

- **20K metabolic features** detected; **10,171 features** used in analysis
- **196** metabolic features significantly associated with malignant glioma ($p < 0.05$); **Figure 1**
- One metabolic feature (**prolyl-hydroxyproline**) was significantly positively associated with glioma (after controlling for FDR at 0.2 level); **Table 2**
- Enriched Metabolic Pathways: **Hexose Phosphorylation, Butanoate Metabolism, Glyoxylate Metabolism; Figure 2**

Figure 1. Metabolite Level Associations

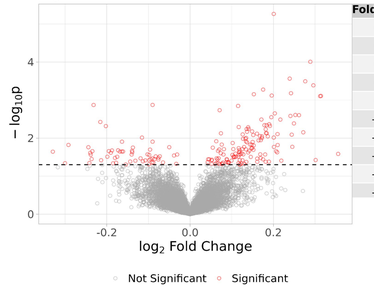
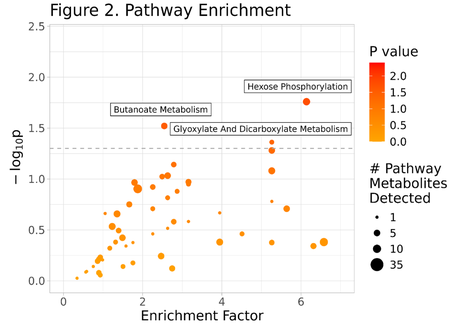


Table 2. Description of Top 5 +/- Metabolic Associations

Fold Change	Std.Error	p-value	Potential Chemicals
0.296	0.0834	4.09e-04	Phenylalanylphenylalanine
0.289	0.0738	9.83e-05	Contains Arginine and Proline
0.276	0.0765	3.20e-04	Contains Isoleucine and Phenylalanine
0.239	0.0654	2.72e-04	Contains Isoleucine and Phenylalanine
0.201	0.0439	5.41e-06	Prolyl-hydroxyproline
-0.090	0.0280	1.34e-03	No Annotation
-0.116	0.0445	9.66e-03	No Annotation
-0.202	0.0716	4.82e-03	Gentiobiosyl 2-methyl-6-oxo-2E4E-heptadienoate
-0.216	0.0742	3.75e-03	Kelampayoside A
-0.232	0.0720	1.34e-03	No Annotation

Figure 2. Pathway Enrichment



- **Additional Analysis:**
  - **1 – 5 Years**: **256** significant metabolic features, 1 enriched pathway: **Di-unsaturated fatty acid beta-oxidation**
  - **5 – 20 Years**: **265** significant metabolic features, 2 enriched pathways: **Xenobiotic Metabolism, Aspartate Metabolism**

DISCUSSION AND CONCLUSIONS

- **Key Interpretations:**
  - Found **elevated prolyl-hydroxyproline levels** in Cases. Proline metabolism is known to be associated with Glioma and is an active area of research **[4]**
  - **Pathway enrichment** discovered in **Hexose Phosphorylation, Butanoate Metabolism, Glyoxylate Metabolism** pathways, which have been previously implicated in Glioma **[5,6]**
- **Study Strengths:**
  - Nested Case-Control Study; **largest sample of Military Service Members for investigation of metabolomics and Malignant Glioma risk, to date**
  - Linear Mixed-Models allow for **analysis of all available samples**
- **Study Limitations**:
  - Metabolic features labeled by annotation algorithms which can **fail to annotate some features**
  - **Histologic subtypes/Cancer stages** known only for cases identified via the DoD Cancer Registry, approx. 45% of cases; precluding analysis due to small sample size
- **Future Work**:
  - Identify **important combinations of metabolites** using dimension reduction and mixture models
  - Use annotations to **identify key classes of metabolic features**, including endogenous metabolites and exogenous exposures such as: PFAS, Plasticizers, etc.
  - Study **environmental exposures** and glioma risk from the LC-MS data
  - Evaluate if **metabolic patterns mediate the relationship** between unique **military environmental exposures and malignant glioma risk**

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