



**BOND
UNIVERSITY**

FACULTY OF HEALTH SCIENCES
& MEDICINE

**Assessment of Immune and Molecular
Changes in T Lymphocytes, Natural
Killer Cells and Vasoactive
Neuropeptides in Chronic Fatigue
Syndrome**

Ekua Weba Brenu HBSc Hon Grad Dip

**Assessment of Immune and Molecular
Changes in T Lymphocytes, Natural
Killer Cells and Vasoactive
Neuropeptides in Chronic Fatigue
Syndrome**

Ekua Webá Brenu

**A thesis submitted in fulfilment of the degree of Doctor of Philosophy
to Bond University**

December 2011

Principle Supervisor: Associate Professor Dr. Sonya Marshall-Gradisnik

Co-Supervisors: Associate Professor Dr. Kevin Ashton &

Professor Mieke van Driel

Abstract

Immune dysregulation due to infection, inflammation or compromises to immune and molecular mechanisms can have detrimental effects on normal physiological functions. Chronic Fatigue Syndrome/Myalgic Encephalomyelitis (CFS/ME) is a disorder of unknown mechanism where diagnosis is often delayed and evidence-based effective treatments are lacking. CFS/ME is characterised by severe fatigue, flu-like symptoms, pain and cognitive disturbances. To date a mechanism explaining the relationship between these symptoms and the physiological irregularities presented by patients remains obscure. As such, CFS/ME patients spend a considerable amount of money on health service cost in pursuit of the most effective treatment to alleviate their symptoms. An important initiative towards better management of CFS/ME is the development of accurate diagnosis. The principal aim of this research is to identify feasible biomarkers that can be used in effective diagnosis of CFS/ME. This research examines the status of lymphocytes in CFS/ME with a specific focus on their cellular function including apoptosis, protein secretion, receptor expression and gene expression. It is hypothesized that irregularities in these cellular processes contribute to the mechanism of CFS/ME and thus may form a suite of diagnostic markers for assessing CFS/ME patients.

Participants for the study comprised 95 CFS/ME patients and 50 non-fatigued controls at baseline. Data for 50 CFS/ME and 27 non-fatigued controls was available for the follow up study at 6 and 12 months. Participants were aged between 25-65 years. The criteria for inclusion were based on the Centre for Disease Prevention and Control (CDC) 1994 clinical diagnostic criteria. Whole blood was collected from each participant at baseline, at 6 months and at 12 months. Flow cytometric protocols were

employed in examining measures of cytotoxic activity in CD8⁺T and Natural Killer (NK) cells, levels of CD56^{bright}CD16^{negative} NK cells CD56^{dim}CD16^{positive} NK phenotypes, CD4⁺T helper cytokine secretion and levels of Foxp3 and vasoactive neuropeptide receptor (VPACR2). The expression pattern of cytotoxic related genes including granzyme A (*GZMA*), granzyme K (*GZMK*), perforin (*PRFI*) and interferon-gamma (*IFN-G*) and the expression of microRNA (miRNA) molecules in NK and CD8⁺T cells were investigated.

At baseline, compared to the non-fatigued controls, CFS/ME patients exhibited significant decreases in cytotoxic activity of NK and CD8⁺T cells, levels of CD56^{bright}CD16^{negative} NK cells and repression of *IFN-G*, *GZMA* and *GZMK* gene expression. At the same time, significant increases in cytokines, IL-10, IFN- γ and TNF- α , FOXP3, VPACR2 and *PRFI* expression were noticed in the CFS/ME patients compared to controls. At 6 months follow up, significant reductions in NK activity, CD56^{bright}CD16^{negative} NK cells, IL-10 and IL-17A were observed in the CFS/ME patients compared to the non-fatigued controls. At 12 months, in contrast to the non-fatigued controls, CFS/ME patients continued to demonstrate significant decreases in NK activity and significant increases in only IL-2. Assessment of miRNA expression revealed a significant down-regulation of a number of miRNAs in CFS/ME patients in comparison to the non-fatigued controls, specifically, *miR-21*, *miR-146a*, *miR-223*, *miR-17-5p*, *miR-103*, *miR-106*, *miR-10a*, *miR-191* and *miR-152* were significantly down-regulated mainly in the CFS/ME patients in comparison to the controls.

In conclusion, the results from this study have elucidated the extent of decreases in cytotoxic activity in CFS/ME. In addition, this study has identified unique immune

related processes and molecules that are compromised in CFS/ME patients. These novel parameters may have important implications in the development of biomarkers for CFS/ME. Moreover the consistent decrease in cytotoxic activity and NK phenotypes over the 12 month period strongly supports their usefulness as biomarkers for diagnosing CFS/ME. These biomarkers if implemented in the clinical setting could potentially assist in improving diagnosis and demonstrating a clear pathomechanism for CFS/ME. Eventually, this may result in the development of better therapeutic and treatment strategies for managing CFS/ME.

Acknowledgements

Firstly, I would like to thank God almighty for his grace during this study. This journey would not have been possible without the tremendous support from my family and friends. I would personally like to thank my mum and dad for all their words of encouragement and the many phone call conversations. My brother and my sister for their emails and letting me know how much fun I was missing miles away from home. My warmest appreciation to my Canadian family; Mawuena for making sure I had some fun aside from studying thank you for organising those amazing trips, Delasie, Barbie and Christine for spending time with me and listening to my woes and Olga, thank you for your phone calls and your prayers. I am grateful to my house mates Rungi and Jessie for bearing with my early mornings and late nights and for being my biggest fans. Kristel, Wen, Sandra, June, Mishal and Joanna, I am most thankful for your support and being a source of distraction when I needed a break from my studies. I would like to thank you for all your words of encouragement.

I would like to thank my supervisors, Dr. Sonya Marshall-Gradisnik, Dr. Kevin J. Ashton and Prof. Mieke van Driel for their support and guidance throughout this project. A special thanks to Dr. Don Staines, Professors Russ Chess-Williams, Chris Del Mar and Paul Glasziou for their contribution and feedback on the manuscripts generated from this project. I would also like to extend my sincere thanks to Tracey Richards, Elizabeth Gordon, Marion Andreas, John Leggett and Shelley Bloomfield for their assistance with my urgent requests for consumables. I would like to convey my gratitude to my team of phlebotomist, Belinda, Ruth, Mandy, Gunn and Rhys for making time in their busy schedules to assist with blood collections from the participants in the study. I would like to express my appreciation to the patients and their families for their patience and most importantly for donating their energy and time to the project.

Table of Contents

Abstract.....	3
Acknowledgements	6
Table of Contents	7
List of Figures.....	14
List of Tables	16
Abbreviations	17
Statement of Novelty	20
Publications from this thesis	21

1. Introduction	26
1.1. Overview of Chronic Fatigue Syndrome	27
1.2. Immunological Parameters.....	32
1.2.1. T Lymphocytes	36
1.2.1.1. CD4 ⁺ T helper Lymphocytes.....	37
1.2.1.2. CD8 ⁺ T Lymphocytes.....	42
1.2.1.3. T Lymphocyte in Chronic Fatigue Syndrome.....	44
1.2.2. Natural Killer Lymphocytes	46
1.2.3. Cytotoxic Activity.....	48
1.2.3.1. Natural Killer Lymphocytes in CFS/ME	52
1.2.4. Soluble Proteins	55
1.2.4.1. CD Molecules and Cytokines in CFS/ME.....	56
1.3. Gene Expression Profile in CFS/ME	61
1.3.1. Cytokine and Chemokine Genes.....	62
1.3.2. Genes Involved in Pathogen Lysis.....	66
1.3.3. Transcription factors	67
1.3.4. Immune Regulators	69
1.4. Immune Regulators as possible markers in CFS/ME.....	73
1.4.1. MicroRNAs.....	73
1.4.2. Vasoactive Neuropeptides	79
1.5. Hypotheses	87
1.6. Aims	88
1.7. Significance.....	89

2. Project.....	90
2.1. Methods.....	91
2.1.1. Participant Recruitment and Follow Up	91
2.1.2. Cell Culture.....	95
2.1.3. Participant Recruitment and Follow Up	95

3. Project One: Immunological Abnormalities as Potential Biomarkers in Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis	97
3.1. Abstract	98
3.2. Introduction	99
3.3. Method	102
3.3.1. Participants.....	102
3.3.2. Sample Preparation and Routine Measurements	102
3.3.3. Assessment of NK Cytotoxic Activity.....	103
3.3.4. Assessment of CD8 ⁺ T Lymphocyte Cytotoxic Activity.....	103
3.3.5. Gene expression in NK and CD8 ⁺ T Cells.....	104
3.3.6. Quantification of NK Phenotypes	105
3.3.7. VPACR2 Stimulation.....	105
3.3.8. Cytokine Determination.....	106
3.3.9. Regulatory T Cell Assessment.....	106
3.3.10. Statistical Analysis.....	106
3.3.11. Ethical Clearance and Participant Selection	107
3.4. Results	107
3.4.1. Lymphocyte Cytotoxic Activity	109
3.4.2. Altered NK Profiles in CFS/ME	111
3.4.3. Profile of CD4 ⁺ T cells Subsets and Protein Expressions.....	113
3.5. Discussion	117
3.6. Conclusions	121

4. Project Two: Longitudinal Investigation of Natural Killer Cells and Cytokines in Chronic Fatigue Syndrome.....	122
4.1. Abstract	123
4.2. Introduction	125
4.3. Method	128
4.3.1. Recruitment.....	128
4.3.2. Data Collection	128
4.3.3. Sample Preparation and Routine Measurements	129
4.3.4. NK cytotoxic activity.....	129
4.3.5. NK subsets	130
4.3.6. T cell specific cytokine distribution.....	130
4.3.7. Statistical Analysis.....	131
4.3.8. Ethical Clearance and Participant Selection	132
4.4. Results	132
4.4.1. Participants.....	132
4.4.2. Longitudinal assessment of NK cytotoxic activity	133
4.4.3. Differential Distribution of NK Cells Between Groups	135
4.4.4. T Cell Related Cytokine Distribution	137
4.4.5. Parameter stability	140
4.5. Discussion	141
4.6. Conclusion.....	145

5. Project Three: Cytotoxic Lymphocyte MicroRNAs as Potential Biomarkers for Chronic Fatigue Syndrome /Myalgic Encephalomyelitis Patients146

5.1. Abstract 147

5.2. Introduction 148

5.3. Method 150

 5.3.1. Subject Recruitment..... 150

 5.3.2. Sample Collection and Cell Isolation 150

 5.3.3. RNA Extraction and cDNA Synthesis of NK and CD8⁺T Cells 151

 5.3.4. RT-qPCR..... 151

 5.3.5. Data and Statistical Analysis 154

5.4. Results 154

 5.4.1. Attributes of Participants 154

 5.4.2. Cell Purity and Recovery 156

 5.4.3. RT-qPCR Results..... 157

5.5. Discussion 163

5.6. Conclusion..... 167

6. Final Discussion and Conclusion.....	168
6.1. Impaired Cytotoxic Activity in CFS/ME	171
6.2. NK Phenotypes.....	176
6.3. Impaired Distribution of immune regulators.....	178
6.4. The Profile of miRNAs in CFS/ME.....	185
6.5. Limitations	192
6.6. Further Research	194
6.7. Conclusion.....	196
7. References.....	198
8. Appendix.....	261

List of Figures

Figure 1: The components of the immune system.	35
Figure 2: Differentiation of CD4 ⁺ T helper cells.	38
Figure 3: Differentiation of CD8 ⁺ T cell.	43
Figure 4: The profile of NK lymphocyte subsets.	48
Figure 5: The mechanism of cytotoxic activity of NK and CD8 ⁺ T lymphocytes.	51
Figure 6: MicroRNA biogenesis and action.	75
Figure 7: VIP and PACAP signalling	84
Figure 8: Criteria for Defining the Various Participant Groups.	94
Figure 9: Selection Process for Experimental Groups.	108
Figure 10: Reduced lytic function of cytotoxic cells in CFS/ME.	110
Figure 11: mRNA Expression of Cytotoxic Molecules in NK and CD8 ⁺ T Cells.	111
Figure 12: Distribution of NK phenotypes.	112
Figure 13: Examination of the expression levels of CD4 ⁺ T cell Related Cytokines in CFS/ME following mitogenic stimulation.	114
Figure 14: FOXP3 expression and CD4 ⁺ CD25 ⁺ T cells in CFS/ME.	115
Figure 15: VPAC2R immune cells in CFS/ME.	116
Figure 16: NK cytotoxic activity was decreased at all time points in the CFS/ME patients.	134
Figure 17: CD56 ^{bright} CD16 ⁻ NK Subset distribution in the CFS/ME group.	136
Figure 18: Between group differences in cytokine production overtime.	138
Figure 19: Overall Cytokine Secretion with Respect to Time.	139
Figure 20: The purity of NK and CD8 ⁺ T cells.	156

Figure 21: Expression profile of non-coding snRNAs in NK cells and CD8⁺T cells
.....158

Figure 22: Expression profile of miRNAs involved in apoptosis.....160

Figure 23: Relative expression data presented as boxplots for miRNAs involved in
cell proliferation in A) NK cells and B) CD8⁺T cells.....161

Figure 24: Relative expression data presented as boxplots for miRNAs involved in
immune function in A) NK cells and B) CD8⁺T cells.....162

List of Tables

Table 1: A summary of studies investigating immune function in CFS/ME and their findings	54
Table 2: Studies investigating cytokines in CFS/ME compared to non-CFS/ME participants.....	60
Table 3: A Summary of Gene expression studies in CFS/ME.....	72
Table 4: MicroRNAs involved in immune related activities	78
Table 5: Characteristics of participants in the present study.....	109
Table 6: Baseline clinical characteristics of chronic fatigue syndrome patients (cases) and non-fatigued controls.	133
Table 7: Summary of miRNA investigated, including their targeted messenger RNAs and function.	153
Table 8: Characteristics of CFS/ME and Non-fatigued Control Participants	155

Abbreviations

AB	Antibody
APC	Antigen presenting cell
AIDS	Acquired Immune Deficiency Syndrome
AU	Australia
cAMP	Cyclic adenosine monophosphate
CCR	Chemokine receptor
CD	Cluster of differentiation
CDC	Centre for disease prevention and control
CFS/ME	Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis
CNS	Central Nervous System
CB4	Coxsackie's B4 virus
CD2BP2	CD2 (cytoplasmic tail) binding protein 2
CTSC	Cathepsin C
CXC	Chemokine
DEFB1	Defensin beta 1
EBV	Epstein Barr Virus
EIF2B4	Eukaryotic translation initiation factor 2B, subunit 4
EIF4G1	Eukaryotic translation initiation factor 4 gamma, 1
FasL	Fas ligand
EGR3	Early growth response 3
FC γ R	Fc gamma receptor
FOXP3	Forkhead/winged helix transcription
GSN	Gelsolin (amyloidosis, Finnish type)

GZMA	Granzyme A
GZMB	Granzyme B
GZMK	Granzyme K
HHPV	Human Herpes Virus
HIF1A	Hypoxia inducible factor 1 alpha
HLA-DR	Human Leuckocyte Antigen
HTLV-1	Human T-lymphotropic Virus Type I
IFN	Interferon
Ig	Immunoglobulin
IL	Interleukin
IL-10RA	Interlukin 10 receptor alpha
IL-6R	Interleukin 6 receptor
IL-6ST	Interleukin 6 signalling transducer
JAK	Janus kinase
LPS	Lipopolysaccharide
LT α	Lymphotoxin alpha
LU	Lytic units
MAPK9	Mitogen-activated protein kinase 9
miRNA	Micro ribonucleic acid
NFKB1	Nuclear factor of kappa light polypeptide gene enhancer in B cells
NFKBIZ	Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor zeta
NK	Natural Killer
PACAP	Pituitary adenylate cyclase activating polypeptide
Prf1	Perforin

RISC	RNA induced silencing complex
RT-qPCR	Reverse transcriptase quantitative polymerase chain reaction
SOCS	Suppressor of cytokine signalling
STAT	Signal transduction and activators of transcription
Th	T helper cell
TGF	Transforming growth factor
TNF	Tumor necrosis factor
TNFRSF1A	Tumor necrosis factor receptor superfamily, member 1A
TRAF	TNF receptor associated factor family of proteins
TRAIL	TNF-related apoptosis inducing ligand
Treg	Regulatory T lymphocytes
US	United States of America
VN(s)	Vasoactive neuropeptide(s)
VIP	Vasoactive intestinal peptide
VPACR	VIP and PACAP receptor

Statement of Novelty

To the best of my knowledge I, Ekua Weba Brenu, declare that this work is an original documentation of the present research. All components of the written work were produced by the candidate unless otherwise specified where due acknowledgement has been given in the form of references.

Ekua Weba Brenu

Publications from this thesis

Journal Publications

Staines, D.R., **Brenu, E.W.**, Marshall-Gradisnik, S.M. 2010. Novel Pathomechanisms in Chronic Fatigue Syndrome/Myalgic Encephalomyelitis. *IACFS/ME Bulletin*, 18, 7-30.

Brenu, E.W., Ashton, K.J., van Driel, M., Staines, D.R., Keane, J., Ramos, S.B., Klimas, N.G., Marshall-Gradisnik, S.M. 2011. Potential Biomarkers for the Diagnosis of Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis. *Journal of Translational Medicine*. 9, 1-9.

Kreijkamp-Kaspers, S., **Brenu, E.W.**, Staines, D.R., Marshall-Gradisnik, S., van Driel, M., (2011). Treating Chronic Fatigue Syndrome: a study into the scientific evidence for pharmacological treatments. *Journal of Australian Family Physician*, 40, 907-912.

Brenu, E.W., van Driel, M., Staines, D.R., Ashton, K.J., Keane J., Hardcastle, S.L., Tajouri, L., Peterson, D., Ramos, S.B., Marshall-Gradisnik, S. 2012. Longitudinal investigation of Natural Killer cells and cytokines in Chronic Fatigue Syndrome. *Journal of Translational Medicine*, 10, 88.

Brenu, E.W., van Driel, M., Staines, D.R., Ashton, K.J., Peterson, D., Marshall-Gradisnik, S. 2012. Cytotoxic lymphocyte microRNAs as potential biomarkers for Chronic Fatigue Syndrome Patients/Myalgic Encephalomyelitis. *Journal of Affective Disorders*, 2012 May 7. [Epub ahead of print].

Book Chapters

Brenu, E.W., Marshall-Gradisnik, S.M. and Staines, D.R. 2010. 'The Immune System in Chronic Fatigue Syndrome/ Myalgic Encephalomyelitis', in E Svoboda &

K Zelenjick (eds), *Chronic Fatigue Syndrome: Symptoms, Causes and Prevention*, Nova Science Publishers, New York, pp.1-25

Brenu, E.W., Atkinson M.G., Ashton K.J, Staines D.R. and Marshall-Gradisnik, S.M. 2011. 'The Genetics of Chronic Fatigue Syndrome', in CR Snell (eds), *Chronic Fatigue Syndrome*. INTECH- Open Access Publisher, Rijeka, ISBN 979-953-307-056-7

Brenu, E.W., Tajouri, L., Staines, D.R. and Marshall-Gradisnik, S.M. 2011. 'Vasoactive neuropeptides in Autoimmune Diseases', in F-P Huang (eds), *Autoimmune Disorders – Current concepts and advances from bedside to mechanistic insights*. INTECH- Open Access Publisher, ISBN 978-953-307-653-9.

Conference Abstracts and Presentations

1. **Brenu, E.W.**, van Driel M., Staines, D.R., Ashton, K.J., Marshall-Gradisnik, S. 2010. G-protein coupled receptors on immune cells in Chronic Fatigue Syndrome. Proceedings of the Seventh International Congress on Autoimmunity, Ljubljana, pp.131.
2. **Brenu, E.W.**, van Driel, M., Staines, D.R., Ashton, K.J., Marshall-Gradisnik, S. 2010. The TH1/TH2/TH17 profile in Chronic Fatigue Syndrome. Proceedings of the Seventh International Congress on Autoimmunity, Ljubljana, pp. 133.
3. **Brenu, E.W.**, van Driel, M., Staines, D.R., Ashton, K.J., Marshall-Gradisnik, S. 2010. Cytotoxic lymphocytes in fatigue related disorders. Proceeding of the Fourteenth International Congress of Immunology, Kobe, International Immunology, vol. 22, pp. i58-i67.
4. **Brenu, E.W.**, van Driel, M., Staines, D.R., Ashton, K.J., Marshall-Gradisnik, S. 2010. Altered CD4⁺T cell profile in Chronic Fatigue Syndrome. Proceeding of

the Fourteenth International Congress of Immunology, Kobe, International Immunology, vol. 22, pp. i58-i67.

5. **Brenu, E.W.**, van Driel, M., Staines, D.R., Ashton, K.J., Ramos, S.B., Keane, J., Atkinson, G., Marshall-Gradisnik, S. 2010. Natural Killer Cell as a Potential Biomarker in Chronic Fatigue Syndrome. Proceeding of the Thirty third Australian Flow Cytometry Group Conference, Sydney, pp. 35.
6. **Brenu, E.W.**, van Driel, M., Staines, D.R., Ashton, K.J., Ramos, S.B., Keane, J., Atkinson, G., Marshall-Gradisnik, S. 2010. NK cells and CD8⁺T cells in Chronic Fatigue Syndrome. Proceeding of the CFS/ME Symposium, Gold Coast, pp. 20.
7. **Brenu, E.W.**, van Driel, M., Staines, D.R., Ashton, K.J., Ramos, S.B., Keane, J., Atkinson, G., Marshall-Gradisnik, S. 2010. T cell subsets in Chronic Fatigue Syndrome. Proceeding of the CFS/ME Symposium, Gold Coast, pp. 20.
8. Kreijkamp-Kaspers S., van Driel M., Marshall-Gradisnik S., **Brenu E.**, Staines, D. 2010. Managing Chronic Fatigue Syndrome (CFS): is there evidence for what patients take? Proceedings of the 2010 Primary Health Care (PHC) Research Conference and Information Service, Australia.
9. **Brenu, E.W.**, Ashton, K.J., van Driel, M., Staines, D.R., Marshall-Gradisnik, S. 2011. Natural Killer Cell as a Potential Biomarker in Chronic Fatigue Syndrome. Proceedings of the NK and NKT Cell Biology: Specificity and Redundancy of Innate Responses Conference. Colorado, pp.112.
10. **Brenu, E.W.**, Ashton, K.J., van Driel, M., Atkinson, G., Staines, D.R., Marshall-Gradisnik, S. 2011. Natural Killer and CD8⁺T cell MicroRNAs in Chronic Fatigue Syndrome. Proceedings of the MicroRNAs in Health and Disease Conference. Alberta, pp. 201.

11. **Brenu, E.W.**, Ashton, K.J., van Driel, M., Staines, D.R., Marshall-Gradisnik, S. 2011. Disparities in innate and adaptive immune cell activities in Chronic Fatigue Syndrome. Proceedings of the Tenth International Association of CFS/ME, Ottawa, pp.29.
12. **Brenu, E.W.**, Ashton, KJ, van Driel, M., Staines, D.R., Marshall-Gradisnik, S. 2011. Longitudinal Assessment of Adaptive Immune Regulation in Chronic Fatigue Syndrome. Proceedings of the Tenth International Association of CFS/ME, Ottawa, pp.30.
13. **Brenu, E.W.**, Ashton, K.J., van Driel, M., Staines, D.R., Marshall-Gradisnik, S. 2011. Expression patterns of miRNAs in lymphocytes Chronic Fatigue Syndrome. Proceedings of the Tenth International Association of CFS/ME, Ottawa, pp.70.
14. **Brenu, E.W.**, van Driel, M., Staines, D.R., Marshall-Gradisnik, S., Peterson, D. 2011. Cytotoxic proteins in Chronic Fatigue Syndrome. Proceedings of the Tenth International Association of CFS/ME, Ottawa, pp.68.
15. **Brenu, E.W.**, van Driel, M., Staines, D.R., Peterson, D., Marshall-Gradisnik, S. 2011. Purinergic Signalling in Chronic Fatigue Syndrome. Proceedings of the Tenth International Association of CFS/ME, Ottawa. Pp. 69.
16. **Brenu, E.W.**, van Driel, M., Kreijkamp-Kaspers, S., Hardcastle, S.L., Keane, J., Peterson, D., Staines, D.R., Marshall-Gradisnik, S. 2011. The effects of vaccination on immune function in Chronic Fatigue Syndrome. Proceedings of the Tenth International Association of CFS/ME, Ottawa.pp.69.
17. Hardcastle, S.L., **Brenu, E.W.**, van Driel, M., Staines, D.R., Kreijkamp-Kaspers, S., Marshall-Gradisnik, S. 2012. Natural Killer cells in patients with

severe Chronic Fatigue Syndrome. Proceedings of the Eighth International Congress on Autoimmunity, Granada.

18. Batovska, J., **Brenu, E.W.**, Ashton, K.J., van Driel, M., Staines, D.R., Marshall-Gradisnik, S. 2012. Expression of inflammatory cytokines in Chronic Fatigue Syndrome. Proceedings of the Eighth International Congress on Autoimmunity, Granada.
19. Marshall-Gradisnik, S., van Driel, M., Ashton, K.J., Hardcastle, S.L., Staines, D.R., **Brenu, E.W.** 2012. Adaptive and innate immune markers as potential biomarkers for Chronic Fatigue Syndrome. Proceedings of the Translational Medicine-2012 Conference, Texas.
20. **Brenu, E.W.**, van Driel, M., Ashton, K.J., Hardcastle, S.L., Staines, D.R., Marshall-Gradisnik, S. 2012. Differential expression of miRNAs in Patients with Chronic Fatigue Syndrome. Proceedings of the Translational Medicine-2012 Conference, Texas.