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Gene Expression in Chronic Fatigue Syndrome

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1. Introduction

Chronic Fatigue Syndrome (CFS) is a disorder of unknown origin likely affecting multiple physiological processes. CFS is often a diagnosis of exclusion following a history of 6 months or more where patients may experience partial to full recovery, relapse or a worsening in symptoms and hence deterioration in health (Brkic et al., 2011). The clinical manifestations include moderate to severe fatigue, muscle pain, swollen lymph nodes, headaches, impaired sleep and cognition (Fukuda et al., 1994). A diagnosis of CFS is made using questionnaires which include Centre for Disease Prevention and control criteria for CFS, the Australian, British and Canadian CFS classifications and the recently developed World Health Organisation's International Classification of Diseases for CFS (Carruthers et al., 2011, Carruthers et al., 2003; Fukuda et al., 1994; Lloyd et al., 1990; Sharpe et al., 1991). CFS is a heterogeneous and multifactorial disorder. Mechanisms to explain the underlying factors and processes that are responsible for disease progression and symptom profile of this disorder remains to be established. However, research has demonstrated that CFS impacts the endocrine, neurological, immune and metabolic processes resulting in impaired physiological homeostasis (Brenu et al., 2010; Demitrack, 1997; Schwartz et al., 1994). While these processes are likely compromised and collectively contribute to ill health in CFS patients, CFS remains a disorder lacking a clear molecular or biochemical cause.

Twin studies have revealed that there is no single genetic factor associated with CFS (Evengard et al., 2005). Several molecular studies have identified genes that are differentially expressed in CFS patients in comparison to non-CFS individuals (Kaushik et al., 2005, Kerr et al., 2008; Gow et al., 2009; Light et al., 2009; Saiki et al., 2008). Additionally, these expressional differences in CFS may be as a result of the multifactorial nature of CFS. The challenge is to understand the relationship between these genetic discrepancies in CFS eventuating discovery of its pathomechanism leading to appropriate treatment and ultimately a cure. Gene expression studies in CFS have shown possible links between CFS and a number of molecular pathways associated with immune, neurological and metabolic processes (Kerr et al., 2008). The purpose of this chapter is to review the literature focusing on gene expression changes and their role in the pathophysiology of CFS.

2. Molecular studies

2.1 Candidate gene studies

Candidate gene studies are mainly employed to address the biological characteristics of known genes that predispose them to have an involvement in CFS. The advantage of this approach is that it allows for the detection of common alleles with some effect on the disease presentation. Comparisons between CFS patients and non-fatigue controls on measures of allele and genotype frequencies of identified markers have shown significant differences between these groups. This method has been used to investigate the human leukocyte antigens (HLA) markers and killer cell immunoglobulin-like markers of NK receptors in CFS patients. In some CFS patients significant increases in HLA alleles, HLA-DQA1*01 and HLA-DQB1*06 have been observed compared to control participants (Smith et al. 2005). Among the killer cell immunoglobulin-like receptors (KIRs), high levels of KIR3DS1 with loss of HLA-Bw4lle80 ligands is common among CFS patients compared to control participants (Pasi et al., 2011). Similarly, other HLA haplotypes such as HLA-DRB1*1301 are elevated in CFS patients (Carlo-Stella et al., 2009). Polymorphisms in other receptors also occurs in CFS, importantly a number of the alleles for the receptor for advanced glycation end product (RAGE) may be decreased in CFS patients (Carlo-Stella et al., 2009). These changes in allelic frequencies and haplotypes especially in the HLA molecules may be associated with the inflammatory state of CFS patients.

Gene studies with SNPs may be an alternative pathway for determining susceptibility to CFS. CFS patients are more likely to have SNP variations for the glucocorticoid receptor gene *NR3C1* with high incidence of risk conferring haplotypes (Rajeevan et al., 2007). The serotonergic system in some CFS patients is compromised and this is typified by an over active 5-hydroxytryptamine (5-HT) and a down regulated hypothalamic-pituitary-adrenal (HPA) axis (Demitrack, 1997). This likely occurs as a consequence of polymorphisms in genes that regulate serotonergic signalling. Hence, in CFS an increase in the polymorphism of the A allele linked with -1438G/A in the *HTR2A* receptor may explain these compromises (Smith et al., 2008). In particular, -1438G/A has been associated with suicide and cognitive impairment (Arango et al., 2003; Reynolds et al., 2006).

2.2 Twin studies

CFS may be prevalent in some families, thus, CFS may have a heritable component. However, the credibility of this observation remains to be determined. Self report measures and restriction fragment length polymorphism are most often used to assess the hereditability of CFS (Crawley & Smith 2007). CFS may have a familial predisposition as relatives of patients with CFS may not necessarily meet the criteria for CFS but may be more prone to experience some of the symptoms of CFS (Walsh et al., 2001). Although twin studies allude to the existence of a genetic predisposition to CFS, this may be higher among monozygotic twins compared to dizygotic twins (Buchwald et al., 2001). Twins with CFS may share similar symptoms and experience the same level of severity in CFS related symptoms (Claypoole et al., 2007). Despite these heritable predispositions observed in twin studies, they are not enough to confirm a genetic basis for CFS (Albright et al., 2011).

2.3 Gene expression microarray studies

Genome wide studies using microarrays is a predictive method of determining genes that may influence unexplained disorders such as CFS for which an aetiological mechanism is lacking. These large scale explorative studies are more often extensive and are able to determine the expression levels of genes expressed in CFS and non-CFS participants. While the results from these studies may be useful, validation through real-time quantitative polymerase chain reaction is most often required to ensure that the identified genes are representative of either a down or an up-regulation in gene expression patterns. Most of these large scale studies have identified genes that are differentially expressed in CFS compared to non-fatigued participants (Cameron et al., 2007; Carmel et al., 2006; Fang et al., 2006; Kaushik et al., 2005; Kerr et al., 2008; Saiki et al., 2008; Whistler et al., 2005; Whistler et al., 2003). In general, these genes regulate important physiological activities that are compromised in CFS. These include immune, endocrine, neurologic, metabolic and cellular activities. Elucidation of genes that predispose an individual to CFS is essential in understanding the mechanism of CFS. Gene expression studies have allowed for the identification of a number of genes involved in different aspects of the disease.

2.4 CFS gene expression studies

Many factors can influence susceptibility to CFS. Changes in the expression of genes important for various physiological processes may affect normal function. The vast majority of research in CFS has confirmed significant compromise to immune, endocrine, neurological and metabolic processes. Immunological abnormalities observed in CFS patients include decreases in cytotoxic activity of Natural Killer (NK) cells and perturbations in cytokine levels.

2.4.1 Cytokine and chemokine genes

Cytokines and their genes are vital for sustaining and regulating innate and adaptive immune activities such as cell differentiation, proliferation and activation. *IL-8* is a pro-inflammatory chemokine gene with chemotactic properties for neutrophils during pathogen invasion and other immunological insults (Huber et al., 1991). In CFS *IL-8* has been shown to be significantly increased in expression in comparison to non-CFS individuals (Vernon et al., 2002). During neutrophil pathogen lysis, phagocytic products are released which acts as a positive feedback process to activate IL-8 to recruit more neutrophils (Ito et al., 2004; Sparkman and Boggaram, 2004). Alterations in *IL-8* mRNA expression is linked with inflammation (Mukaida, 2003; Nozell et al., 2006; Xie, 2001). An increase in *IL-8* expression noted in CFS patients may occur as a result of an increase in oxidative stress during inflammation (Shono et al., 1996; Ito et al., 2004; Sparkman and Boggaram, 2004). The promoter region of *IL-8* is bound and activated by transcription factors including NF-κB A substantial decrease in the expression of *NF-κB* negatively affects *IL-8* (Huang et al., 2001). *NF-κB* is a necessary component in the activation and signalling pathway of other leukocyte cytokines and reductions in their expression increases vulnerability to infectious agents and inflammatory reactions (Artis et al., 2003; Bohuslav et al., 1998; Sha et al., 1995; Campbell et al., 2000; Yang et al., 1998).

During inflammation, immune cells such as macrophages produce pro-inflammatory molecules such as TNF-α. The severity of the inflammatory response determines the level of TNF-α produced. The *TNFA* gene is contained within the MHC complex; once it has been translated it functions by binding to TNF receptors TNFR1 or TNFR2. TNF-α has a higher affinity for the TNFR2 receptor compared to the TNFR1 (Orlinick and Chao, 1998). TNFR2 modulates the proliferation of T lymphocytes and encourages pro-inflammatory responses. Usually a low concentration of TNF-α is required to activate TNFR2 while TNFR1 is stimulated in the presence of increased concentration of TNF-α. These interactions are vital for cell death signalling, cytotoxicity or cellular apoptosis (Zhou et al., 2002). TNFR1 and TNFR2 compete for TNF-α (Bodmer et al., 2002). *TNFA* is instrumental in controlling and regulating viral infection, NF-κB signalling, neuropathic pain and cytokines (Lee et al., 2009). In the central nervous system (CNS), glial-derived *TNFA* modulates synaptic plasticity by increasing the expression of *AMPA* and also reducing long-term potentiation in the hippocampus (Leung and Cahill, 2010; Orlinick and Chao, 1998; Pickering et al., 2005). *TNFA* expression increases in the presence of stress and this has been observed in CFS patients although this increase was similar in healthy controls (Light et al., 2009). While mRNA levels in *TNFA* may be similar in CFS and healthy controls, polymorphism within *TNFA* may affect their ability to perform efficiently as shown in other diseases (Zhang et al., 2010).

IFNAR1 is required for IFNα/β antiviral responses and is therefore a key component in immunity against viral and bacterial infections (David, 2002). CFS patients are known to have significant increases in viral antigens and these may persist where the activities of IFNs are ineffective in inducing antiviral immune responses (Bansal et al., 2011). In CFS, *IFNAR1* is increased in expression (Kerr et al., 2008) and this may occur as a result of persistent viral antigens or viral infected cells. It has been observed that *IFNAR1* tends to increase in the presence of infections such as Human papillomavirus (HPV) and influenza (Gius et al., 2007; Jia et al., 2010). *IL10-RA* is both down- and up-regulated in CFS patients (Kaushik et al., 2005; Kerr et al., 2008). The protein, IL10-Rα is expressed on T cells, B cells, monocytes, macrophages, dendritic cells, NK cells, mast cells and microglia with no intrinsic kinase activity. Interactions between IL10-Rα and IL-10 stimulate the phosphorylation and activation of JAK1 and TYK2 kinases (Hebenstreit et al., 2005; O'Shea et al., 2002). This sequentially phosphorylates tyrosine residues in the cytoplasmic regions of IL-10Rα chains and forms docking sites for STAT3 (Moore et al., 2001). Janus Kinases and signal transducers and activators of transcription (JAK/STAT) pathways are essential for regulating cytokine mediated responses and *vice versa* (Schindler, 1999; Schindler et al., 2007). Genes such as *STAT5A* are induced by cytokines IL-2, IL-4 and IL-7. *STAT5A* is a critical element in the proliferation and survival of Th2 cells (Hebenstreit et al., 2005; Lin and Leonard, 2000). Differential expression in *STAT5A* in CFS likely affects the Th1-Th2 cytokine balance, possibly favouring an anti-inflammatory/Th1 like immune response, while suppressing pro-inflammatory immune reactions (Ihle, 2001; Kagami et al., 2001; Saiki et al., 2008; Skowera et al., 2004).

JAK1 contains cytoplasmic tyrosine kinases that react in a non-covalent manner to a varying number of cytokine receptors and is therefore implicated in lymphocyte development in particular, lymphocyte proliferation and differentiation (Flex et al., 2008). *STAT5A* and *JAK1* are requisite for IL-2, IL-10, IL-7, IL-9, IL-13, IL-22 and IFN-α signalling (Schindler et al.,

2007). Hence, over expression of both *STAT5A* and *JAK1* (Kerr et al., 2008; Saiki et al., 2008) may substantially alter the normal function of these cytokines and their receptors. These may include IFN-α, IL-7 and IL-10 (Kerr, 2008). Such adverse effects may cause shifts in the inflammatory profile causing either an increase or decrease in pro- and anti-inflammatory cytokines (Gupta et al., 1997; Vojdani et al., 1997). The exact profile of cytokines in CFS remains to be determined, although, a number of studies suggest that CFS is characterised by a predominant anti-inflammatory immune state (Skowera et al., 2004) others advocate a pro-inflammatory immune profile (Swanik et al.). This mixed picture suggests dysregulation of the balance in pro- and anti-inflammatory mechanisms.

Bidirectional communication between JAK/STAT signalling and cytokines is important for maintaining immune homeostasis. For example, IL-6 binds to its receptor and positively stimulates a number of JAKs and STATs which initiates a sequence of downstream effects that prompt the development and maturation of progenitor cells (Kamimura et al., 2003; Kristiansen and Mandrup-Poulsen, 2005). However, the expression of *IL-6* can be dampened by suppressors of cytokine signalling (SOCS), this inevitably increases inflammation (Croker et al., 2003; Zhang et al., 2008). Hence, differential expression in *IL-6*, *IL6R* and *IL6ST* (Kerr, 2008; Light et al., 2009) may have adverse consequences on the activity of IL-6 in both the innate and adaptive immune response. This may also affect *JAK1* in CFS (Guschin et al., 1995). Therefore in CFS differential expression in cytokine, JAK and STAT genes may increase susceptibility to prolonged immune deterioration.

TNFRSF1A is the gene for pro-inflammatory tumour necrosis factor (TNF)-α receptor, which increases pro-inflammatory events and stimulates the generation of cytokines through the activation of NF-κB (Nowlan et al., 2006). *TNFRSF1A* is also involved in cell death pathways involving TNFR-associated factor (TRAF) domains (Baud and Karin, 2001). In some CFS patients, cell death is particularly increased in neutrophils in comparison to non-fatigued controls (Kennedy et al., 2004; See et al., 1998; Vojdani et al., 1997). NF-κB gene, *NFKB1*, is decreased in expression in some CFS patients. Decreases in both *NFKB1* and *TNFRSF1A* in CFS may potentially affect the proliferation of cytokines and chemokines such as IL-8 (Kerr, 2008). Additionally, NF-κB is inhibited by *NFKBIZ* which is also down regulated in CFS (Kerr, 2008). In the immune system, NF-κB is activated in response to toll-like receptors (TLR) (Kitamura et al., 2000; Yamazaki et al., 2001) by *TRAF3* (Hauer et al., 2005; He et al., 2007; He et al., 2006). *TRAF3* is instrumental in T cell related immune responses (Goldfeld et al., 1991). *TRAF3* and *NFKBIZ* are collectively involved in the downstream activities of TNFRSF1A and NF-κB. Modifications in these genes can affect other cytokine pathways.

Another important gene, *HIF1A,* which encodes for the hypoxia induced transcription factor HIF1α, is responsible for the induction of apoptosis and inhibition of cell proliferation (Akakura et al., 2001; Carmeliet and Tessier-Lavigne, 2005; Yu et al., 2004). *HIF1A* also regulates pathogen lysis or phagocytosis mediated by neutrophils and macrophage (Nizet and Johnson, 2009). Oxidative phosphorylation is an important component of the phagolytic mechanism. This is deficient in some CFS cases and may cause a decrease in the amount of reactive oxygen species released from neutrophils to effectively breakdown the phagocytosed pathogen (Brenu et al. 2010). Impairments in oxidative phosphorylation in CFS patients may ensue from a downregulation in *HIF1A*.

As previously mentioned, chemokines such as IL-8 are important soluble proteins that are necessary for immune cell trafficking during infection and other inflammatory insults. Chemokines such as CXCR4 are expressed by neutrophils, monocytes and T lymphocytes and their activities are regulated by cAMP, IL-6, IL-4, IL-10 and reactive oxygen species (Jazin et al., 1997). CXCR4 is necessary for hematopoietic cell trafficking, differentiation, endothelial migration and cell proliferation in the CNS and immune systems (Jazin et al., 1997; Moepps et al., 1997; Zou et al., 1998). *CXCR4* is another gene involved in the identification of microbial factors such as LPS. The CXCR4 protein is part of the seven trans-membrane G-protein super family of receptors (Pierce et al., 2002). *CXCR4* promotes the proliferation of tumour cells via the MAP/ERK pathway and can in some cases have anti-apoptotic properties (Darash-Yahana et al., 2004). Similar to the *TLR4* and *CD14* in response to LPS, *CXCR4* expression becomes upregulated (Moriuchi et al., 1998). As these genes were simultaneously measured in the same CFS population, it is possible to posit that in some cases of CFS there are high levels of LPS factors, in particular LPS factors that cause heightened persistent immune activation. In these individuals perhaps these immune activations are not cleared and therefore encourage the survival of these microbial pathogens in circulation for a longer duration. In some CFS patients, *CXCR4* is upregulated (Gow et al., 2009; Kerr, 2008) which may suggest an altered chemokine profile in CFS patients. Other genes such as *CD47* are present on cells in the CNS and immune system. CD47 is a necessary factor in the migration of neutrophils and other cells (Brown et al., 1990; Gao et al., 1996; Lindberg et al., 1993; Parkos et al., 1996). It is also important in T cell activation and neurological function such as memory (Ticchioni et al., 1997; Waclavicek et al., 1997). In CFS, lymphocyte numbers in circulation may vary from patient to patient, however, the available number of lymphocytes at sites of infection or engaged in eliminating infected cells is not known. Incidentally, an upregulation in chemokine genes *IL-8*, *CXCR4* and *CD47* may affect the efficiency of these cells to migrate to areas of infection (Gow et al., 2009; Kerr, 2008).

As previously discussed *TGF-β1* is an important pleiotropic cytokine as it regulates peripheral tolerance mechanisms in response to injury, cell growth and survival (Marie et al., 2005). *TGF-β1* is a critical component of the Treg differentiation pathway in particular Treg survival and FOXP3 expression (Marie et al., 2005). *TGF-β1* is also an important factor in cellular apoptosis involving Fas mediated apoptotic pathways and oxidative phosphorylation (Sanchez-Capelo, 2005). An upregulation in this gene may stimulate pathways that increase spontaneous apoptosis in neutrophils (Kennedy et al., 2004) and thereby prevent the induction of oxidative stress in CFS individuals (Brenu et al., 2010).

2.4.2 Genes involved in pathogen lysis

An important mechanism employed by both NK and $CD8^+$T cells to lyse viral pathogens is cytotoxic activity. The end result of cytotoxicity is cell death or apoptosis. Cytotoxic activity is achieved when the NK or $CD8^+$T cells release lytic granules containing granzymes and perforin into the target cell through exocytosis (Leong & Fehinger 2010). In the cell membrane of the infected cell perforin facilitates the binding of granzymes to different organelles of the cell and induce either caspase dependent or independent apoptosis (Pradelli et al., 2010). *GZMA* is the gene for granzyme A, it is essential for natural cytotoxic activity and antibody dependent cytotoxic activity of $CD8^+$T and NK cells via FCγRII (CD16)

receptor (Lahmers et al., 2006; Madueno et al., 1993). GZMA induces slow apoptosis once released into the target cell. In some CFS patients mRNA levels for *GZMA* and *GZMB* are low while levels of perforin are increased (Brenu et al., 2010; Saiki et al., 008). Differential expression in these lytic molecules may explain the inefficiency of NK or $CD8^+$T cells in CFS patients to effectively execute cytotoxicity in the presence of pathogenic cells (Kilmas et al., 1990; Maher et al., 2005; Brenu et al., 2011).

However, discrepancies in the cathepsin C (*CTSC*) gene, which has wide distribution throughout the human body particularly in myeloid cells, polymorphonuclear leukocytes, alveolar macrophages and osteoclasts, can potentially affect the effectiveness of lytic cells (Hakeda and Kumegawa, 1991; McGuire et al., 1997; Rao et al., 1997b). Deficiencies in *CTSC* are associated with impaired activation of GZMA and GZMB in NK and cytotoxic T lymphocytes (Pham and Ley, 1999). This implies that CFS patients presenting with atypical *CTSC* expression may also exhibit decreased GZMA and GZMB production (Maher et al., 2005; Saiki et al., 2008). Cytotoxic activity may be considerably low as a consequence of low expression of granzyme genes in CFS patients, thus an increase in viral load will be highly detrimental to the compromised immune system.

2.4.3 Transcription factors

The regulation of genes is dependent on molecules known as transcription factors (Farnham, 2009). In CFS, transcription factor genes are differentially expressed. Among them is *EGR3,* which regulates lymphocyte proliferation, apoptosis and inflammatory responses (Beinke and Ley, 2004; Inoue et al., 2004; Jiang et al., 2005). *EGR3* in T lymphocytes stimulates Fas-L formation and cytotoxic activity of $CD8^+$T lymphocytes (Matsuoka and Jeang, 2005). Their dysregulation affects the production of IL-2 (Safford et al., 2005), an important factor in anti-inflammatory Treg and Th2 differentiation. In CFS patients this may be associated with the deficits in cytotoxic activity and the presence of anti-inflammatory immune responses (Kerr et al., 2008).

TRAIL is another gene expressed by both innate and adaptive immune cells. It is important in inducing cellular apoptosis in immune cells, monocytes, dendritic cells, NK and $CD8^+$T lymphocytes (Schaefer et al., 2007). In cytotoxic cells such as NK and $CD8^+$T, TRAIL serves as an alternative pathway for effective cytotoxic activity against viral antigens (Janssen et al., 2005; Kayagaki et al., 1999). Th2 cells preferentially express TRAIL and therefore are able to kill other immune cells and infected cells (Zhang et al., 2003). Hence, CFS patients with deficiencies in this gene may experience decreases in NK and $CD8^+$T cell cytotoxic activity and induction of apoptosis, making them more vulnerable to immune infection and hindering normal immune function in these individuals.

NFATC1 is the gene for the nuclear factor of activated T lymphocytes belonging to the NFAT family of transcription factors. This transcription factor regulates genes encoding cytokines and cytokine receptors in response to antigen activation (Crabtree and Clipstone, 1994; Rao et al., 1997a). Importantly, they are implicated in T cell abundance, Th2 differentiation and cytokine production (Yoshida et al., 1998; Ranger et al., 1998). Impaired Th2 cytokines in some cases of CFS may emanate from perturbed expression in *NFATC1*. Conversely, other genes such as human β-defensin 1 *(DEFB1)* may have unfavourable consequences on the Th1 cytokines causing an over abundance of these proteins in some

cases of CFS (Wehkamp et al., 2005). *DEFB1* is involved in immunomodulation against microbial peptides in both the innate and adaptive immune response. Using the CCR6 receptor they are able attract dendritic cells and $CD4^{+}$T lymphocytes (Yang et al., 1999) during infection and inflammation (Dommisch et al., 2005; Sun et al., 2005; Wehkamp et al., 2005). Animal models have confirmed that an increase in susceptibility to microbial infections infections ensues in the event where *DEFB1* is deficient or mutated (Morrison et al., 2002; Moser et al., 2002). CFS related serological and virological studies indicate significant increases in viral antigens in some CFS patients and this may also be linked to defects in *DEFB1*.

ETS1 encodes for a transcription factor that binds to DNA sequences with an invariant GGA (Gegonne et al., 1993). *ETS1* like many other transcription factors is upregulated in CFS patients (Kerr et al., 2008). *ETS1* is an early response transcription factor gene with binding sites for transcription factors AP1, AP2 and ETS at its promoter end (Dittmer, 2003; Thomas et al., 1997). It is found in the nucleus where phosphorylation of Ras strongly increases transcriptional activity of ETS1 and its interactions with other proteins through the ETS1 domain (Wasylyk et al., 1998). *ETS1* acts together with other genes to increase its function hence it is positively regulated by AML-1, Pit-1 and HIF-2α (Dittmer, 2003). *ETS1* sequentially excites the DNA binding process of these genes . ETS1 can be inhibited by CAMKII, Daxx/EAPI and ZEB (Dittmer, 2003). ETS1 synergises with TGF-β to activate other genes. Activated T cells usually have a decreased expression of *ETS1* compared with dormant T cells T cells (Bhat et al., 1990). *ETS1* is found in T, B, and NK cells. It is a proto-oncogenic transcription factor which is involved in naïve T cell development and differentiation (Di Santo, 2010). In T cells, deficiencies in *ETS1* can inhibit T cell responses to other stimulatory signals and increase susceptibility to cell death. Although, *ETS1* expression decreases in the activated T cells in the developing T cell it is essential in prompting the expression of *TCRa* and *TCRβ* (Giese et al., 1995). Additionally, *ETS1* interacts with other immune regulators such as STAT5 which is implicated in T cell responses (Rameil et al., 2000). *ETS1* is an essential gene necessary for the optimal optimal development of naïve T cells, an increase in this gene may suggest an increase in resting T cells over activated T cells in CFS patients. Although, increases in some subsets of T cells such as FOXP3 Tregs (Brenu et al., 2011b) have been suggested, it is possible that these cells are not adequately activated and a majority of these cells are in the resting phase it is most likely thus are not able to effectively clear infections or encourage most favourable immune profile in CFS patients. NK decrease in cytotoxic activity may also be related to *ETS1* over expression as *ETS1* is important in NK cell development (Yokoyama et al., 2003). Failure of NK cells to develop into efficient lytic cells can hinder their ability to recognise and eliminate pathogens. Loss of function in ETS1 impairs proper lymphocyte differentiation and permits autoimmune responses (Wang et al., 2005). However, FOXN1 is involved in the development and differentiation of thymic epithelial cells (TECs) (Su et al., 2003). The expression of *FOXN1* is controlled by Bone Morphogenetic proteins (BMPs) and WNT (Coffer and Burgering, 2004). Immune deficiencies arise when mutations occur in *FOXN1* (Coffer and Burgering, 2004). In CFS, *FOXN1* has been suggested as a potential candidate gene for the development of biomarkers for CFS and may be linked to the severity of CFS (Presson et al., 2008). Abnormal changes in *FOXN1* affects T cell development and function and may relate to the cytokine pattern in CFS.

The histone acetyltransferase and deacetylase (*HDAC7A*) gene modulates nuclear histone acetylation. It inhibits the activity of myocyte enhancer-binding factor (MEF) and is highly expressed in thymocytes (Kasler and Verdin, 2007). This gene is responsible for transcriptional repression and the maintenance of cellular integrity (de Ruijter et al., 2003). It is an efficient co-repressor of the androgen receptor (AR) (Karvonen et al., 2006). It regulates apoptosis in developing thymocytes and may be associated with the decrease cytotoxic activity noticed in some CFS patients. Given that transcription factors are important in most cellular processes, a decrease or increase in its expression can have crucial consequences on the normal functioning of many physiological processes.

2.4.4 Immune regulators

The current data on CFS strongly support an impaired immune function characterised by differential expression of cytokines and decreases in cytotoxic activity. These observed immune defects may ensue from changes in the expression of certain genes involved in the signalling pathways of these immune indices. *MAPK9* codes an important signalling molecule known as the JNK2 protein kinase and its disruption is associated with the pathogenesis of destructive insulitis (Jaeschke et al., 2005). Some microbes are able to downregulate MAPK9 which in turn inactivates JNK2 thereby decreasing transcriptional events in this pathway (Zhang et al., 2004).

The cytochrome P450 (*CYPIBI*) gene has a role in responding to environmental toxins and mutagenic products (Hayes et al., 1996; Shimada et al., 1996). Although it is expressed in higher concentrations in breast cancer (Huang et al., 1996), in CFS it most likely involved in increased susceptibility to toxic agents. As CFS is likely a multi-factorial disorder, prolonged exposure to toxic agents may predispose an individual to CFS. *CMRF35/CD300C* encodes the CD300c leukocyte surface protein present on macrophages (Turnbull and Colonna, 2007). Secretion of TNF-α and IFN-α is highly dependent on *CYPIBI* (Ju et al., 2008). Additionally, abnormalities in CFS cytokine profiles possibly occurs where *CMRF35* is differentially expressed, distorting anti-viral (IFN- α) and pro-inflammatory (TNF-α) activities required for maintaining immune homeostasis (Sen, 2001).

Adhesion molecules are important for interactions between T cells and other cellular surfaces. In T cells the adhesion molecule CD2 allows T cells to connect with other cells. CD2 is regulated by *CD2BP2* (the CD2 binding protein 2) which increases binding specificity of the cytoplasmic domain of the T cell adhesion molecule CD2 and localizes it to the cell membrane and nucleus. TLR4 is an anti-tumour repressor and which inhibits the destruction of tumour antigens in lysosomes of dendritic cells. This facilitates antigen presentation to T cells and enhances the binding of LPS to MD-2. TLR4 mediated signalling can either occur via MyD88 dependent or independent pathway. When the MyD88 dependent pathway is used, this leads to the production of pro-inflammatory cytokines while the MyD88-independent pathway induces Type I interferons and interferon inducible genes (Lu et al., 2008). Human macrophages express CD14, a glycosylphosphatidylinositol-linked plasma-membrane glycoprotein, on their cell surfaces that facilitate the induction of apoptosis of foreign cells (Vita et al., 1997). *CD14* in conjunction with *TLR4* and *MD2* initiates the formation of a lipopolysaccharide receptor complex that controls immune responses to pathogens in the respiratory system, recognition of LPS and the generation of systemic inflammation (Wright et al., 1990). An increased expression in both *TLR4* and *CD14*

may suggest an increase in LPS, LPS increases the expression of these genes (Foster et al., 2007). The biphasic expression of these genes allows them to have either an activating or a limiting effect on other genes. Additionally, in most cellular responses to bacterial infection due to LPS release, the MyD88-independent signalling pathway is activated. TLR4 may bind to the cell membrane allowing efficient presentation of LPS to TLR4. It is evident that modulation of the expression of *CD14* and *TLR4* can have severe consequences on the ability of immune cells to recognise microbial particles. Nonetheless, these observations are indicative of a heightened immune activation as a possible contributory factor to the compromised immune function in CFS patients.

Other neutrophil related genes have also been suggested to be differentially expressed in CFS patients. Genes such as *SNAP23* (Synaptosomal-associated protein 23) and *CFACAM8* are upregulated in some cases of CFS (Gow et al., 2009; Kerr et al., 2008). *SNAP23* is present mostly in non-neuronal tissues and is part of the t-SNARE complex (Washbourne et al., 2002). *SNAP23* controls neutrophil exocytosis and also cell surface granule interactions and is thus essential for intracellular trafficking of vesicles/granules (Lacy, 2006; Zylbersztejn and Galli, 2011). *CFACAM8* on the other hand is important in cell adhesion, migration and signal transduction in neutrophils (Zhao et al., 2004). These genes are therefore essential for the movement of neutrophils to sites of inflammation and or infection.

2.4.5 Other cellular processes

Other genes examined in CFS are necessary for many cellular processes. These genes may be implicated in functional properties of cells in a number of physiological processes suggesting a heterogeneous clinical presentation. For example, *ARPC5* is the smallest subunit of the actin related protein complex 5, which controls the polymerization of actin (Pollard, 2007). This normally occurs in response to cellular motility during the polymerization of new actin filament. Dendritic cells have not being adequately investigated in CFS, however, their morphogenesis may be compromised as evident by the over expression of *ANAPC11* (Gumy et al., 2011). *ANAPC11*, anaphase promoting complex subunit 11, has a role in dendritic cell morphogenesis (Domingo-Gil et al., 2010). It is part of a complex that targets and degrades proteins during mitosis. The migration of cells from one point to another, in circulation, involves the interplay of a number of genes such as A*TP5J2*, an ATP synthase involved in cellular processes requiring ATP (Cheung and Spielman, 2009). *APP,* the amyloid precursor gene is a marker for Alzheimer's disease (Zetterberg et al., 2010). It regulates cell surface proteins (Hoe and Rebeck, 2008).*GSN* is an anti-apoptotic regulator, and an actin serving protein that modulates actin assembly, disassembly and regulates cell motility via the actin network (Hoe and Rebeck, 2008). *REPIN1* is highly expressed in the liver and adipose tissue. It is a replication initiator and is involved in a number of metabolic disorders (Bahr et al., 2011).

A number of genes identified in CFS patients are involved in metabolic pathways specifically the protein kinases, ATP and cAMP related genes. These genes interact to maintain normal metabolic activity. These include transmembrane protein 50A (*TMEM50A)* located in RH gene locus, *ATP6V1C1* which regulates extracellular acidification to facilitate bone resorption (Feng et al., 2009) and *PRKAR1A* inhibits protein phosphorylation and tumour development (Bossis and Stratakis, 2004; Groussin et al., 2002). Mutations in *PRKAR1A* have been associated with tumour development (Scott, 1991; Tasken et al., 1997).

AKAP10 is the kinase-anchoring gene 10 which is currently an identifier for determining the risk of developing colorectal cancer (Wang et al., 2009a; Wang et al., 2009b). It also requires cAMP to diffuse through the cytoplasm to propagate its signal. *AKAP10* modulates immune responses related to PGE2/EP2/cAMP/PKA pathway (Kim et al., 2011). It targets regulatory subunit of PKA to specific cell sites such as the mitochondria. The cAMP responsive element binding protein (*CITED2)* refers to (Xu et al., 2007). It modulates hypoxia inducible factor dependent expression of vascular endothelial growth factor and hematopoietic stem cells. In CFS, we have recently reported an increase in neuropeptide receptors, specifically in VPACR2 in a cohort of CFS patients (Brenu et al., 2011b). This increase in VPAC2R may translate into an increase in cAMP causing a potential increase in PKA activity in CFS. An increase in cAMP may increase the expression of *PKAR1A*, *AKAP10* and *CITED2* and hence making their regulatory effects redundant and altering the physiological homeostasis. Tyrosine kinase non-receptor 2 (*TNK2)* functions as a translational repressor during cell fate specification and is necessary for the expression of epidermal growth factor receptors (Howlin et al., 2008).

Mitochondria related genes are also differentially expressed in CFS these genes include *SUCLA2, MRRF, EIF4G1, MRPL23*, *GABPA*, *PRDX3* and *EIF3S8*. As cellular function is impaired in CFS it is likely that important organelles especially those related to metabolic processing may be functioning at suboptimal levels. *SUCLA2*is involved in mitochondria regulation (Miller et al., 2011), *EIF4G1* is an initiation factor implicated in mitochondrial induced apoptosis (Bushell et al., 2000), *MRRF* regulates cell survival (Rorbach et al., 2008) while *PRDX3* prevents oxidative damage to cells (Ejima et al., 2000). Additionally, *GABPA*, *EIF3S8* and *MRPL23* have broad functions in mitochondria (Wyrwicz et al., 2007; Zhang and Wong-Riley, 2000). Mitochondria in the muscles of patients with CFS produce relatively low energy when compared to non-fatigued controls (Plioplys and Plioplys, 1995). In some cases patients may present with structural deformities in the mitochondria, these include subsarcolemmal mitochondrial aggregates, compartmentalization of the internal mitochondrial membrane and polymorphism (Plioplys and Plioplys, 1995). Similarly defective mitochondrial metabolic activity may be characterised by the presence of neurotoxic phospholipids and phospholipids of mitochondria that appear after microbial infections (Hokama et al., 2008). Neutrophil in the innate immune system employ respiratory burst and oxidative phosphorylation as a means to effectively kill and clear pathogen invasion. This unique mechanism is advantageous and reduces the persistence of microbial infections. Respiratory burst in CFS is flawed. The authors have previously shown that in CFS neutrophils are able to recognise and engulf pathogens however, the ability to induce and activate reactive oxygen species to induce respiratory burst is significantly compromised when compared to non-fatigued controls (Brenu et al., 2010). Incidentally, abnormal mitochondrial function exists in CFS where ATP and oxidative phosphorylation is substantially lower in the CFS patients (Myhill et al., 2009).

2.4.6 Neurology and endocrine function

Neurological dysfunction in CFS may present in many formats, the most obvious documented symptoms are loss in memory and concentration, sleep disorder and severe headaches. While the exact cause of CFS remains to be determined it has been postulated that neuroimmune abnormalities in form of dysregulation in cytokines due to a prevalent

viral antigens in the brain may enhance CFS related neurological deficits (Kuratsune et al., 2001). In CFS a number of genes that regulate neurological and endocrine function have also being detected to be equivocally expressed when compared to non-fatigued controls. These observations may relate to the impairment in cognition and other neurological functions associated with this disease. The HPA axis is distorted in CFS and this may have a bearing on the changes in other genes (Ursini et al., 2010). EIF2B4 affects neurological function and has been shown to be related to mitochondrial function. It refers to the eukaryotic translation factor 2B subunit 4. It has been implicated in Vanishing White Matter disease (VWM). Although CFS is not an inherited disorder it may share similar symptoms with VWM. Both CFS and VWM are associated with infections (Bansal et al., 2011). CFS patients and patients with VWM may demonstrate abnormalities in cerebrospinal fluid (Schutzer et al., 2011a; Schutzer et al., 2011b). White matter studies in CFS are inconsistent, in some instances, abnormal white matter has been observed (Lange et al., 1999; Schwartz et al., 1994). Also, grey matter in some CFS patients may be reduced (de Lange et al., 2005). These confounding factors may to some extent relate to the severity of neurological impairments in patients with CFS.

NHLH1 is the helix-loop-helix transcription factor whose expression is restricted to the nervous system. It is important during development and neuronal differentiation (De Smaele et al., 2008). In mice loss of *NHLH1* generates irregular autonomic function characterised by arrhythmia, dampening of parasympathetic and in increase in death (Cogliati et al., 2002). A number of CFS patients may present with a dysfunctional autonomic system which may be related to an increase in heart rate and a decrease in systolic blood pressure. Additionally, irregularities in pH and heart rate variability occur in CFS patients following exercise (Jones et al., 2009; Newton et al., 2007). SORL1 refers to the sortilin-related receptor. It is a neuronal sorting protein-related receptor that is involved in intracellular trafficking. It directs trafficking of amyloid precursor protein and is decreased in the brains of humans suffering with Alzheimer's disease (Shibata et al., 2008). It is associated with risk of late onset of AD. This gene may be partially responsible for the memory loss experience by some CFS patients although this needs further clarification (Reynolds et al., 2010). *PKN1* is part of the neurofilament head rod domain kinase. It is a serine/threonine protein kinase that mediates cellular response to stress (Kato et al., 2008). *PKN1* regulates gene expression in response to extra cellular stimuli. Overexpression of *PKN1* causes a substantial elevation in the phosphorylation of ERK (Kajimoto et al., 2011). A number of CFS patients show an upregulation in genes in the ERK signalling pathway when compared to non-fatigue controls (Kerr et al., 2008). Phosphorylation of TRAF1 is dependent on *PNK1* and this also regulates the ratio of TRAF1 and TRAF2 and determines the NF-κβ and JNK signalling (Kat et al., 2008). TRAF1 and TRAF2 in turn modulate the signalling activity of IKK and JNK (Gotoh et al., 2004). An upregulation in *PKN1* may severely alter the downstream signalling pathways associated with PKN1. Importantly NF-κβ immune related activities maybe distorted where PKN1 is upregulated. NF- κβ regulates inflammatory cytokines (Park and Levitt, 1993). In CFS alterations in cytokine distribution has been observed. This may be either towards a pro- or anti-inflammatory cytokine profile. In the CNS system shifts in cytokine profiles have been reported for many autoimmune disorders and a similar mechanism may occur in CFS patients as a consequence of prevailing viral and microbial antigens that are not effectively cleared following infection. Perhaps these antigens remain and therefore modulate the cytokine milieu in the CNS.

Additionally heightened pro-inflammatory mechanisms followed by an increase in suppression may exist in the CNS neuroimmune system in an attempt to dampen viral and microbial survival in the CNS.

During development, *HOXA1* is expressed in the hindbrain (Studer et al., 1998). It is an essential developmental gene belonging to the homeobox genes. It is associated with autism. The product generated from translation of this gene is a transcription factor which is important in cell differentiation, embryogenesis, defining body plan during development and oncogenic transformation. Recently *HOXA1* has been observed to be a target of miR-10a (Shen et al., 2009). *COMT* is the catechol-O-methyltransferase, it is critical for the metabolic degradation of dopamine (Blanchard et al., 2011). It is involved in the function of dopamine in the prefrontal cortex of the human brain thus it is involved in frontal lobe functioning (Meyer-Lindenberg et al., 2005). The inability of most CFS patients to concentrate for long periods on activities requiring higher order cognitive function may be explained by dysregulation in *COMT*.

2.5 MicroRNA

MicroRNAs (miRNA) are recently described, highly conserved molecules with regulatory activities in multi-cellular organisms such as mammals. They are small components of ribonucleoprotein particles belonging to a family of RNA which have diverse effects on physiological function. MicroRNAs are suppressors of gene expression and affect either translational processes or the stability of mRNAs through the encouragement of decay processes, deadenylation and decapping processes termed RNA interference (Mishima et al., 2006; Wu et al., 2006). The expression of the miRNA gene results in the creation of the primary transcript (pri-miRNA) that is 60-80 nucleotides in length. This pri-miRNA contains a hairpin stem-loop structure which is cleaved by the enzyme Drosha (RNA III enzyme) and DGCR8 (DiGeorge critical region 8), resulting in the creation of a structure comprised of a ~22 base pair stem, 2-nucleotide 3′ overhang and a loop, collectively known as the precursor-miRNA (pre-miRNA) transcript (Lee et al., 2003). The pre-miRNA transcript is transported into the cytoplasm where RNase III enzyme, Dicer, cleaves the terminal loop of the pre-miRNA transcript to form a 18-24 base pair product (Lee et al., 2002). A currently unidentified helicase then produces individual miRNA strands – a mature miRNA, which is the mediator of mRNA repression, and the passenger strand, which is rapidly degraded. The mature miRNA is integrated into an RNA induced silencing complex (RISC) with Argonaute (Ago) proteins where it is further processed (Khvorova et al., 2003; Lee et al., 2003; Lingel et al., 2003; Mourelatos et al., 2002). The final product formed from this sequence of events is a miRNA-RISC complex. Suppressive effects of miRNA on mRNA molecules occur via the RISC complex in which Ago is able to exercise endonuclease activity on the double stranded miRNA-mRNA structure (Hutvagner and Zamore, 2002). The mature miRNA can bind to complete and incomplete complementary strands of mRNA molecules and degrade the mRNA or inhibit translation respectively (Behm-Ansmant et al., 2006; Hutvagner and Zamore, 2002; Lim et al., 2005). Through these mechanisms it has been extensively documented that miRNA regulates a diverse range of physiological activity and also contributes to disease states such as cancer (Lu et al., 2005) and cardiomyopathy (Chen et al., 2006). Interactions between the miRNA and mRNA molecules are important for maintaining physiological processes in development and homeostasis and have already

been associated with numerous disease states. However, the role of miRNA in CFS is largely unknown. For further reading on the cellular and physiological processes of miRNA, the reader is directed to Sun et al. (2010).

With consistent trends between immunological dysfunction and CFS becoming more apparent, miRNAs related to immune function are relevant to this understudied area and may hold potential for treatment. The first study of its kind to assess miRNA expression in CFS investigated the expression of miRNAs relating to immune function, apoptosis and cell cycle regulation (Brenu et al., 2011a). This study identified a general down regulation in most of the miRNA transcripts in NK cells of CFS patients. This supports the observation of immune dysregulation in CFS patients (Brenu et al., 2010; Maher et al., 2005), however, whether this is linked to a decrease in miRNA processing activity or is specific to miRNA function is yet to be determined. More specifically, this study found decreases in miRNA transcripts that are involved in apoptosis. CFS patients have been shown to demonstrate significant decrease in cytotoxic activity of NK cells hence decreases in miRNAs may contribute to the pattern of NK cytotoxicity noticed in CFS patients. For example, miR-146, which mediates the expression of NFκ-β and thus the transcription of numerous inflammatory mediators, was significantly decreased in CFS (Brenu et al., 2011b). The consequence of this may be a decrease in the cytokine secretion by NK cells as NFκ-β is an important regulator of cytokine production in these cells (Gerondakis & Siebenlist 2010). Incidentally *IFN-γ* was noticed to be significantly decreased in expression in the same cohort of CFS patients with a decrease in miR-146 (Brenu et al., 2011b). Similarly, in the presence of an altered NFκB expression, NK responsiveness to IL-12 in CFS patients may be dampened compromising immune response to both infection and homeostasis (Broderick et al., 2010). Further studies are needed to verify whether miRNAs contribute or are linked to depressions in IL-8, IL-13 and IL-5 and increased activity of IL-1α, IL-1β, IL-4, IL-5, IL-6 and IL-12 in CFS patients (Fletcher et al., 2009). Substantial decreases in the expression miR-21 were observed in the CFS patient group. These results suggest the presence of a possible compromise in the maturation and function of lymphocyte translating into decreases in cytotoxic activity (Salaun et al., 2011). Direct evidence of this however, remains to be established.

At the present miRNA research is at its infancy hence the exact role of miRNAs in NK cells is subject to speculation. Similarly the gene expression miRNA studies in CFS is severely lacking therefore only postulations can be made about the link between the miRNAs and the disease. However, the promising data shown in the aforementioned studies likely suggest that miRNAs may indeed play greater roles in the dysregulation of immune function in CFS.

MicroRNAs may regulate other aspects of immune function in CFS, the above mentioned study is limited as it only examines NK and $CD8^+$T cells. However miRNAs are known to regulate most if not all immune cells. In the innate immune system, miRNAs such as miR-155 enhance the maturation of macrophages and dendritic cells via the TLR receptor pathway, causing heightened sensitivity in these cells to antigens in circulation (O'Connell et al., 2007; Tili et al., 2007). $CD4^+$ T cell matuation into various subsets in the periphery is regulated by miRNAs (Wu et al., 2007). The generation of Tregs that express FOXP3 is to some extent dependent on miRNAs (Kohlhaas et al., 2009). Any perturbed effects in miRNAs can influence thymic and peripheral derived Tregs especially in response to TGF-β stimulation on naive

$CD4^+$ T lymphocytes (Ha, 2011). Modulation of the effects of these molecules is essential for appropriate immune response to bacterial and viral invasion and current studies show these areas may be impaired in CFS sufferers. Importantly, deficiencies in components of the miRNA such as Dicer promotes a predominant Th1 response governed by IFN-γ with a reduction in the effects of Th2 cells and Treg cells (Cobb et al., 2006). In contrast a predominant Th2 $CD4^+$ T cell profile prompting systemic inflammation emanates from deficiencies in the miR-155 (Rodriguez et al., 2007; Thai et al., 2007) while in the absence of miR-101, autoreactive T cell mediated autoimmunity occurs (Yu et al., 2007). In CFS there are inconsistencies in the data on Th1/Th2 profiles. It is likely that in the event that immune related miRNAs are differentially expressed, shifts in Th1 and Th2 inflammatory response and defects in TLR signalling may occur, and this may be related to the pathophysiology of CFS. Whilst it is believed that many miRNAs are yet to be discovered, evidence is scarce to describe the multitude of various physiological roles of currently discovered miRNAs. Despite this, the current evidence that links miRNA dysregulation to the characteristics of CFS has shown that there is merit in the roles of miRNA in CFS. Further advancements are needed to characterise the role of miRNAs in CFS.

Our current investigative techniques for identifying transcriptional changes in known miRNAs are quickly advancing through microarray technology. This method uses the same principle as DNA microarray technology and allows for semi-quantitative expression changes of a large number of miRNAs in a single chip (Li and Ruan, 2009). The clear advantages of using microarray is the high throughput and vast number of transcripts analysed in a single chip as compared to low throughput and tedious methods of microRNA cloning, northern blotting and real time RT-qPCR. As mentioned in section 2.2, this gives investigators the power to identify expression differentials in gene categories, allowing the association of a particular state or disease to a molecular or physiological category. Numerous limitations are associated with microarray technology, most importantly is the ability to identify changes in already known miRNAs, as the targets require hybridisation with specifically designed probes attached to the chip. Moreover, these expression changes are only semi-quantitative due to the hybridisation techniques used, resulting in a lack of reproducibility. These pitfalls are similar to those in DNA microarray but are likely not as pronounced due to the various isoforms and large size of genes as compared with miRNAs (Fathallah-Shaykh, 2005). Finally, microarray technology has the disadvantage of only being able to detect known transcripts. With possibly many undiscovered transcripts this poses a problem for miRNA discovery in differential expression using this method and may also interfere with target specificity. However, more recent investigative techniques look promising for the discovery of new target miRNAs as well as addressing many of the pitfalls of the low-throughput and microarray based methods. One such example is sequence-by-synthesis technology, which has recently been used with investigative application and is likely to be used more widely in the near future (Morin et al., 2008).

2.6 MicroRNA-based gene therapy

The roles of microRNAs in diseases are likely to become targets for therapy. The current experimental practice is known as gene silencing and the specialised transcripts used in such instances are known as small interfering RNA (siRNA) (Wang et al., 2011). The cellular method of translation or transcription repression is the same as miRNAs – through the use of RISC, however in gene silencing the target-specific substrate, the siRNA, is exogenously

introduced. There is currently little in the way of clinically translated practice of gene therapy using the siRNA method, as it is associated with a number of problems. The most notable of these is delivery and cell specific targeting. The current means of delivery in experimental models is via adeno- or adeno-associated virus constructs transcribing the specific siRNA or siRNAs of choice. To allow for cell specific targeting certain virus constructs are suited for various cell types however the lack of specificity and low percentage uptake makes this is an improbable method of therapy at present. There are however new experimental means of delivery currently being optimised (Yuan et al., 2011). Once such issues have been addressed the significance of gene silencing may be relevant in CFS. With increasing amounts of evidence indicating that CFS likely has a strong molecular basis, such methods hold merit once initial targets have been discovered. The current stance on miRNAs in CFS calls for further research in the area in both genome wide miRNA analysis in longitudinal studies, and also the search for new miRNAs possibly implicated in this disorder. With the current technology available, and promising experimental therapeutics such as gene silencing, miRNA is likely to play a large and significant role in possibly the development of biomarkers, mechanisms or treatment of CFS.

2.7 Future directions

The high variability in genomic anomalies within CFS patients may be an underlying cause of our current inability to effectively treat the disorder. No specific conditioning or dieting routine has proven beneficial for a wide majority of patients and even more elusive are effective pharmacological targets for this population. It is probable that various underlying mechanisms may give rise to the variable patient-described symptoms of CFS. This may explain the lack of efficient treatment options and opens questions in the area of pharmacogenomics. Pharmacological agents specific to genetic traits that are associated with CFS and possibly subsets of the disease may be useful in monitoring CFS. In the context of CFS, this pertains to our lack of understanding and inability to define areas of treatment, suggesting that a suitable treatment may call for the definition of subtypes of the disease or populations that are genetically predisposed to such symptoms.

However, at present the most important aim of research worldwide is to establish biomarkers for CFS. Currently the most stable and reliable marker is NK cytotoxic activity (Brenu et al., 2011b; Fletcher et al., 2009; Klimas et al., 1990; Maher et al., 2005). Consistent data worldwide suggest that a decrease in cytotoxic activity is a hallmark of CFS. In most cases this decrease has been associated with differential expression in cytotoxic molecules including *GZMA, GZMK, GZMB* and *PRF1* (Brenu et al., 2011b; Saiki et al., 2008). Developing pharmacological agents that specifically target these cytotoxic genes in order to increase or decrease their expression might be an alternative method of treating impaired cytotoxic activity in CFS patients. Subtypes of CFS patients may exist and this may be based on clusters of symptoms or severity of illness. Hence these may need to be considered when developing appropriate agents for modulating the disease.

3. Conclusion

In summary, the repercussions of these changes gene expresseion may contribute tremendously to the disease profile of CFS. The genes discussed above have vital roles in most immune related activities such as inflammatory modulation, lymphocyte and cytokine

activation, lymphocyte differentiation and proliferation and are also implicated in the apoptosis signalling pathways. Hence, an up-regulation in chemokine genes may affect leukocyte response to infection and other immunological insults while down-regulation in pro-inflammatory cytokine genes may disrupt inflammatory reactions. Importantly, the consistent observation of impaired NK cytolysis in CFS is partly due to the reduced expression of perforin and granzymes genes. As previously discussed these granzymes induce apoptosis of antigens within the cell. Variation in cytokine release and production can be explained by the altered levels of pro- and anti-inflammatory cytokines. Most of these cytokines are engaged in other physiological processes. Hence, defects in their production can severely hinder physiological function and homeostasis. Other symptoms such as cognitive impairment and changes in the HPA axis in CFS patients may emanate from an increase *NHLH1* while changes in mitochondria genes contribute to fatigue and muscle weakness. Although, these studies have to some extent provided information on the genetics of CFS patients, it is not known whether CFS elicits these changes in gene expression patterns or *vice versa*. Similarly, most of the genes observed in these studies have not been replicated in other CFS patients. It is therefore very difficult to ascertain which specific cells are compromised among the CFS population. Further studies are now required to determine how changes in gene expression can be related to the mechanism of CFS and the specific cells or systems that may be severely compromised in this disorder.

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