Neurological disorders represent one of the leading global causes of disability. They include both age-related neurodegenerative disorders such as Parkinson’s disease (PD), as well as neurodevelopmental disorders including schizophrenia (SCZ). In the past several decades, researchers have made significant progress in unravelling the pathological mechanisms of these disorders. This has been accelerated by the merging of various scientific disciplines and an enhanced interaction between basic, applied, and translational research in what is now widely known as the field of neuroscience. This integrative field has allowed for researchers to study diseases from different perspectives and to use different strategies for discovery, understanding or treatment. Our recent review on molecular chaperones illustrated how studying this specific set of proteins and their roles in abnormal protein folding, oxidative stress, and mitochondrial function may reveal their greater impact on a range of central nervous system disorders. Specifically, we discussed the diverse roles of a novel molecular chaperone protein called Catecholamine-
CRP40: A novel molecular chaperone with implications in neurological diseases

In part one of our two-part review on molecular chaperones as potential targets in neurological diseases (Lubarda 2013a), we outlined the roles of heat shock proteins in diseases characterized by abnormal protein folding. We presented research done by our laboratory at McMaster University, led by Dr. Joseph Gabriele, on the novel molecular chaperone Catecholamine-regulated protein 40 (CRP40). CRP40 was discovered during investigation of proteins with dopamine (DA) binding abilities and purported to be involved in the DA signalling cascade through its interactions with DA and co-localization with tyrosine hydroxylase, the critical enzyme in DA synthesis (Gabriele 2009). CRP40 was found to belong to a family of heat shock proteins involved in protein folding and maintenance of mitochondrial function and homeostasis — conditions that are impaired in various neurological disease states. It was discovered that CRP40 is an alternative splice variant of the 70kDa heat shock protein, mortalin, which has been linked to Parkinson’s disease (PD) through the following evidence: mortalin is expressed from a putative PD locus on chromosome 5; concentrations of mortalin are reduced in post-mortem PD patients; mutations in the mortalin gene, in regions from which CRP40 is expressed, have been found in German and Spanish PD patients—these mutations have been associated with impairments in mitochondrial function and increased neuronal susceptibility to oxidative damage (Burbulla 2010).

In part one of our review on CRP40, we also revealed evidence for the possible involvement of this protein in neurological disorders such as schizophrenia (SCZ). CRP40 is dysregulated in human post-mortem SCZ brain samples. In part two of our two-part review, we present our newest evidence for the genetic dysregulation of CRP40 in the blood of live SCZ and PD patients. These compelling findings link CRP40 to central nervous system disorders and add significant value to the important global task of identifying valid, reliable, and simple biomarkers for definitive and early diagnosis of these debilitating disorders.

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Dr. Joseph Gabriele, on the novel molecular chaperone Catecholamine-regulated protein 40 (CRP40). CRP40 was discovered during investigation of proteins with dopamine (DA) binding abilities and purported to be involved in the DA signalling cascade through its interactions with DA and co-localization with tyrosine hydroxylase, the critical enzyme in DA synthesis (Gabriele 2009). CRP40 was found to belong to a family of heat shock proteins involved in protein folding and maintenance of mitochondrial function and homeostasis — conditions that are impaired in various neurological disease states. It was discovered that CRP40 is an alternative splice variant of the 70kDa heat shock protein, mortalin, which has been linked to Parkinson’s disease (PD) through the following evidence: mortalin is expressed from a putative PD locus on chromosome 5; concentrations of mortalin are reduced in post-mortem PD patients; mutations in the mortalin gene, in regions from which CRP40 is expressed, have been found in German and Spanish PD patients—these mutations have been associated with impairments in mitochondrial function and increased neuronal susceptibility to oxidative damage (Burbulla 2010).
We designed methods to measure CRP40 mRNA levels using the versatile technique of real-time reverse-transcriptase polymerase chain reaction (RT-PCR) in the blood of live PD and SCZ patients. CRP40 was dysregulated in both of these cases in comparison to healthy, normal controls and negative controls (Alzheimer’s disease, stroke) (Groleau 2013, Lubarda 2013b). These findings point to the possibility of distinct roles of CRP40 in central nervous system disorders. In this review, we discuss the implications of CRP40 in SCZ and PD, and present evidence that may advance our understanding of these complex disorders and open up novel avenues for investigation of heat shock proteins as potential biomarkers.

CRP40: Implications in Schizophrenia

The pathogenesis of SCZ, though still incompletely understood, is instigated by oxidative stress and mitochondrial dysfunction (Bitanihirwe 2011, Ciobica 2011, Martins-de-Souza 2011, Wood 2009, Yao 2001, Yao 2011). Oxidative stress is caused by reactive oxygen species (ROS), natural by-products of mitochondrial energy metabolism. If ROS are not controlled, they can react with important cellular components, leading to irreparable damage like neural degeneration.

Exacerbated ROS production in conjunction with dysfunctional energy metabolism has been observed in human tissue samples from SCZ patients (brain tissue, blood, and cerebrospinal fluid) (Prabakaran 2007, Schwarz 2008). The neurodegeneration observed in SCZ has been associated with genetic variations of heat shock proteins (HSPs) with specific implications in oxidative stress and apoptosis including 70kDA HSPs (HSP70) (Kim 2008, Schon 2003). Since Mortalin and its splice variant, CRP40, show significant homology to HSP70, they have emerged as proteins of interest to researchers studying SCZ.

Many studies done over the last decade have revealed CRP40 as a key player in SCZ. Using human post-mortem SCZ samples obtained from the Stanley Foundation Neuropathology Consortium, western blot analysis revealed a significant decrease in the protein expression of CRP40 in the ventral striatum of SCZ patients compared to healthy subjects (Gabriele 2005, Gabriele 2010a). Non-medicated schizophrenic patients showed an even greater reduction in CRP40 expression than medicated schizophrenics when compared to controls (Gabriele 2005). The effect of antipsychotic drug use on CRP40/mortalin mRNA levels in schizophrenic post-mortem samples was also investigated using human SCZ dorsolateral prefrontal cortex brain samples (Gabriele 2010a). CRP40/mortalin mRNA expression was assessed via real-time RT-PCR, revealing a positive correlation between lifetime antipsychotic drug use and increased CRP40/mortalin mRNA expression in SCZ patients (Gabriele 2010a).

In further studies, CRP40/mortalin were underexpressed by gene knockdown in the medial prefrontal cortex in rats (Gabriele 2010b). Sensorimotor gating was measured by prepulse inhibition (PPI), which assesses the startle reflex response to acoustic stimuli. Deficits in PPI are typically observed in patients diagnosed with SCZ. Significant deficits in PPI were found in the antisense oligodeoxynucleotide treated group when compared to the control group (Gabriele 2010b).

Importantly, it has been observed that haloperidol, a DA receptor agonist and antipsychotic drug used regularly in the treatment of SCZ, causes significant upregulation of CRP40 at the striatum, but not in the frontal cortex (Gabriele 2007, Gabriele 2009, Gabriele 2010a, Sharan 2001). Haloperidol blocks binding of DA to D2 receptors, causing increased free, unbound DA. This excess DA causes CRP40 upregulation (Gabriele 2007, Gabriele 2010a, Sharan 2000). Similarly, amphetamine, a DA agonist used in a rat model of SCZ, causes significant upregulation of CRP40 at the striatum (Gabriele 2002). These findings support the hypothesis that anti-psychotic drugs and psychostimulants like cocaine affect CRP40 protein levels in the mesocorticolimbic brain regions and further reinforce its involvement in these psychotic disorders (Gabriele 2007, Sharan 2001, Sharan 2003).

Most recently, CRP40 has been found dysregulated in human blood samples of patients with SCZ (Groleau 2013). CRP40/mortalin mRNA was analyzed in white blood cells of first episode schizophrenia subjects, chronic/treated schizophrenia subjects and healthy controls. Significant reductions in CRP40/mortalin mRNA were found among first episode schizophrenia subjects and chronic schizophrenia subjects compared to healthy controls (Groleau 2013). These results suggest a possible functional role of CRP40 in the pathogenesis of schizophrenia, as well as the potential for future diagnostics based on the CRP40 protein.
CRP40: Implications in Parkinson's disease

Parkinson's disease (PD) is a movement disorder characterized by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNc) (Lang 1998, Schapira 1999). The resulting loss of DA neurotransmission is associated with impairments in motor and locomotion (Alberts 1965). Researchers have linked mitochondrial dysfunction and oxidative stress to PD pathophysiology (Lang 1998, Schapira 1999). In the SNc, free DA is oxidized to ROS known as Quinones (Stokes 1999). Since natural oxidation of DA leads to the formation of this potent oxidant species, it is of particular interest to PD research. As a protective measure, the human brain employs molecular chaperone proteins to defend against oxidative stressors (Becker 1994, Kaul 2007). PD patients show impaired function of key mitochondrial proteins, many of which are molecular chaperones (Becker 1994, Kaul 2007). Many have reported the possibility of combined effects of increased oxidative stress, impaired mitochondrial function, and dysregulation of molecular chaperone proteins in the pathogenesis of this movement-related disease.

Recent work has exposed the likely role of the CRP40 chaperone in the pathology of Parkinson's disease. Genetic analysis of a population of PD patients found two previously unidentified missense mutations of Mortalin/CRP40 in some patients with PD that were completely missing in any control subject (De Mena 2009). Interestingly, mutations in the mortalin gene found in PD patients are located within the C-terminal domain of the protein, from which CRP40 is expressed (Burbulla 2010, Sklar 2004). Since mortalin is involved in mitochondrial health, a mutation of this calibre could be responsible for the cellular stress that leads to degeneration like that seen in PD. Mortalin has been found to interact with PD-implicated proteins such as DJ-1 and α-synuclein (Jin 2006, Jin 2007). A missense mutation in Mortalin, like those described above, would certainly have a negative effect on these important protein interactions (De Mena 2009, Jin 2006, Jin 2007). Among studies using human post-mortem brain samples, Mortalin/CRP40 were found to be underexpressed in patients with PD compared to controls (Jin 2006, Shi 2008). Specifically, there is a quantitative decrease in Mortalin/CRP40 expression with progression of the disease (Shi 2008). In a study using rats with intrastriatal injections of 6-hydroxydopamine and reserpine treatment (models of PD), results showed decreased levels of CRP40 in the striatum (Modi 1996).

Most recently, CRP40 has been found dysregulated in human blood samples of patients with PD (Lubarda 2013b). CRP40 mRNA was analyzed in platelets of Parkinson's disease subjects and healthy controls. Differences were observed between health states with reduced CRP40 expression among PD subjects compared to controls (Lubarda 2013). These results suggest that blood levels of CRP40 are congruent with post-mortem findings showing CRP40 is genetically dysregulated in PD, and that CRP40 exhibits potential as a biomarker and in future diagnostics for Parkinson's disease.

CRP40: A Future Diagnostic Solution for Neurological Disorders?

The challenges of diagnosing neurological disorders are well known. There is a lack of valid and reliable biomarkers for early detection, specifically those that could monitor disease progression and efficacy of treatments. For instance, diagnosis of SCZ is currently based on clinical criteria and despite extensive research, biomarkers are still lacking (Tandon 2008). Our findings point to the possibility of utilizing CRP40 as a blood biomarker for SCZ. However, more extensive research is required to explore the pathophysiological heterogeneity that is observed in SCZ and to determine whether CRP40 can be used to explain some of the neurobiological processes and clinical expression of the intermediate phenotypes of this complex disease.

Similar strategies can be applied to studying PD as well. The notion of PD as a motor disorder has been altered to encompass several non-motor features, and prodromal symptoms such as hyposmia, rapid-eye-movement behaviour disorder, constipation, and depression (Siderowf 2012). There is now a paradigm shift in research that focuses on diagnosing PD in prodromal stages; however, biomarkers are sparse (Siderowf 2012). Currently, several of the available biomarkers for PD are based on DA transporter imaging, but the technologies are highly costly, somewhat invasive, and not yet widely available (Siderowf 2012). Further, DA imaging techniques may not be ideal for prodromal detection as the development of DA deficiency may be preceded by other events, such as aberrations in protein folding (Siderowf 2012). For these reasons, investigation of molecular chaperone proteins that regulate protein homeostasis, such as CRP40, could possibly be used to diagnose diseases in early stages. We are currently conducting a large-scale study with funding from the Quebec Consortium for Drug Discovery, in association with AstraZeneca, Merck and Pfizer, to validate the potential use of CRP40 as a biomarker for PD. This initiative could lead to the development of highly specific, sensitive, and more affordable diagnostic tests. As well, this research may herald possibilities for the development of disease-modifying therapies based on restoration of function of essential heat shock proteins.
References


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