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Pharmacogenomics of chemotherapy induced cognitive dysfunction

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PHARMACOGENOMICS OF CHEMOTHERAPY INDUCED COGNITIVE DYSFUNCTION

by

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An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
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Thesis Supervisor: Associate Professor Daryl J. Murry

ABSTRACT

Cognitive decline is increasingly recognized as a side effect of chemotherapy. However, cognitive decline doesn't occur in all patients receiving chemotherapy, and there is variability in the cognitive domains affected (*Ahles; JCO,Oct 20, 2012:3675-3686)*. Safety pharmacogenomics, i.e. using genetic variations to predict response/toxicity, offers an exciting approach to identify the subset of patients most likely to suffer from cognitive decline post chemotherapy. Consequently specific therapeutic interventions can be developed to target this group of patients, and/or alternate chemotherapeutic regimens can be used to limit toxicity, thereby offering a way to individualize therapy while minimizing toxicity.

In our research we studied the effect of 16 SNPs in 6 genes on cognition in a sample of healthy older adults. We found that SNPs that affect serotonin, dopamine and glutamate levels in the brain influence cognition in a healthy sample of older adults, possibly in a domain specific manner. This allowed us to identify a group of healthy adults who inherently have lower cognitive functioning in some domains but that is still within the normal range. In addition individuals with SNPs that previously were associated with lower levels of myeloperoxidase performed better on the executive functions, verbal memory, verbal IQ and IQ. SNPs associated with lower levels were also associated with improvement in self reported verbal and visual memory post chemotherapy. APOE E2 allele was associated with higher cognitive performance compared to other alleles. However we didn't see an effect of APOE post chemotherapy.

In chapter five, the effects of 31 SNPs in 15 genes on cognition post chemotherapy were evaluated in community dwelling lymphoma patients. Changes in the domains of verbal memory, visual perceptual memory, and attention of the Multiple Ability Self Report Questionnaire were observed following chemotherapy, but only when groups were stratified by genotype. Contrary to what we might expect, patients showed

improvements in function after chemotherapy. However, using patient stratification based on genotype, specific groups of patients had a measurable decline in cognitive function post chemotherapy. Interestingly a SNP in the DNA replication enzyme and the target of doxorubicin topoisomerase II was associated with varying degree of self reported attention; specifically the AA genotype of rs471692 was associated with statistically significant decline in attention post chemotherapy. This indicates that cognitive changes following chemotherapy can be subtle, and stratification by genotype helps us in identifying susceptible individuals and provides some insights on the inconsistencies that are frequently reported in the literature.

These results allow for identifying genetic risk factors associated with chemotherapy-induced cognitive changes, which will ultimately help in developing therapeutic approaches for the management of those deficits. Strategies to avoid chemotherapy-induced cognitive changes will be prospectively evaluated in future studies and include alternative chemotherapy and less toxic regimens, intervention strategies to improve cognitive abilities, and drug therapy to improve cognition in patients who develop chemotherapy-induced cognitive changes. The overarching goals of our studies are to help improve cancer patients' quality of life while maintaining or improving cancer cure rates.

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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This is to certify that the Ph.D. thesis of

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To my beloved parents, Fahad AlEjielat and Lamia Sunna, and to my sister and brothers, Rana, Iyad, Emad and Zeyad, for their continued love and support

Jesus looked at them and said, “With man this is impossible, but with God all things are possible.”

Matthew 19:26
The Bible, New International Version (NIV)

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improvements in function after chemotherapy. However, using patient stratification based on genotype, specific groups of patients had a measurable decline in cognitive function post chemotherapy. Interestingly a SNP in the DNA replication enzyme and the target of doxorubicin topoisomerase II was associated with varying degree of self reported attention; specifically the AA genotype of rs471692 was associated with statistically significant decline in attention post chemotherapy. This indicates that cognitive changes following chemotherapy can be subtle, and stratification by genotype helps us in identifying susceptible individuals and provides some insights on the inconsistencies that are frequently reported in the literature.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

INTRODUCTION

In a large patient population, a medication that is proven efficacious in many patients often fails to work in some other patients. Furthermore, when it does work, it may cause serious side effects, even death, in a small number of patients (1). Personalized or individualized medicine is often defined as the tailoring of therapies to defined subsets of patients based on their likelihood to respond to therapy or their risk of adverse events(2). The purpose is to optimize the benefit and/or limit the harm of interventions (3). The idea and practice of personalized medicine is not new. Using insulin preparations for the treatment of diabetes mellitus, titrated based on blood sugar level is a form of individualization. But perhaps the most evident form of individualization is therapeutic drug monitoring (TDM) (3). In TDM initial doses/dosage regimens are determined based on previously known information such as a defined therapeutic range and patient characteristics. The regimen can be adjusted later based on blood or plasma concentrations and/or response. TDM requires prior knowledge of the pharmacokinetic parameters and pharmacodynamics of the drug (3). In the recent years and after the sequencing of the human genome, pharmacogenomics became an important part of individualization. Genetic variations in genes involved in drug transporters, metabolizing enzymes, and drug targets can influence response (1). Detecting these variations can be used to guide therapy. An example of such case is the use of thiopurine methyl transferase (TPMT) status to guide therapy by 6 mercaptopurine. TPMT catalyzes the S-methylation of 6-mercaptopurine, azathioprine, and thioguanine, to inactivate the thiopurine drugs, which are used for the treatment of leukemia and autoimmune diseases. More than 20 variant alleles of the TPMT gene have been identified, among which TPMT*2, TPMT*3A, and TPMT*3C are defective alleles that produce poor enzymatic

activities. Approximately 90% of whites inherit high enzyme activity, 10% inherit intermediate activity (heterozygous), and 0.3% inherit low or no activity. Persons carrying defective TPMT alleles accumulate higher levels of cytotoxic thiopurine nucleotides than those with the wild-type alleles after receiving a standard dose of the drugs, leading to severe hematological toxicity by the parent drugs. In these scenarios, a reduced drug dose should be prescribed (1). Another example is the use of expression status of drug targets. The monoclonal antibody trastuzumab (Herceptin) was designed to target only tumors with HER2 overexpression, and women diagnosed with breast cancer are now routinely tested for HER2 expression before the start of treatment to identify those who would benefit from receiving the drug(3). Another important area of using pharmacogenomics is safety. Safety pharmacogenomics is the application of pharmacogenomics for the purpose of avoiding significant drug induced side effects in selected patients(3). An example of this is the use of HLA-B*5701 genotyping to predict hypersensitivity reactions in patients with HIV-AIDS before treatment with abacavir (4).

Cognitive decline is increasingly recognized as a side effect of chemotherapy (5). But it doesn't occur in all patients, and there is variability in the cognitive domains affected, as well as the degree of decline (5). Safety pharmacogenomics offers an exciting approach to identify the subset of patients most likely to suffer from cognitive sequelae post chemotherapy. Consequently specific therapeutic approaches can be developed to target this group of patients, and/or alternate chemotherapeutic regimens can be used to limit toxicity, thereby offering a way to individualize therapy.

In our research, we reviewed the current status of literature regarding the effect of chemotherapy on cognitive function. In addition we reviewed some cognition-related candidate genes. We presented what is currently known about the cognitive effects of single nucleotide polymorphisms (SNPS) in those genes in healthy individuals as well as cancer patients. Then we determined the influence of some of those SNPS on cognition in a sample of healthy older adults. And finally, we determined the influence of certain

SNPs on cognitive function post chemotherapy in a sample of community dwelling lymphoma patients.

We hypothesized that genetic variations in candidate genes will have an influence on cognitive function in healthy adults. Specifically, genetic variations in genes that affect the levels of the neurotransmitters in the prefrontal cortex, i.e. serotonin, dopamine, and glutamate, will influence cognition. In addition, genetic variations that lead to an increase in oxidative stress, and lower levels or functionality of the efflux pump P-gp across the blood brain barrier, and carriers of the E4 allele of Apolipoprotein E will have worse cognitive performance. Myeloperoxidase genotype will be used as the marker for oxidative stress.

To test this hypothesis we evaluated the cognitive performance of healthy older adults using standardized neuropsychological tests in relation to genetic variations. This work is summarized in chapter 4.

Secondly we hypothesized that genetic variations will modulate the effects of chemotherapy on cognition. Specifically, genetic variations that adversely affect cognitive function in healthy individuals will also predispose to having poorer function post chemotherapy. In addition, genetic variations in genes across the drug disposition pathway such as transporters, metabolizing enzymes, and DNA repair genes will influence drug levels and functionality and affect its delivery to the brain, and affect cognition and frailty among lymphoma survivors.

To test this hypothesis we evaluated neurocognitive abilities and frailty among community living non-Hodgkin's lymphoma patients who received doxorubicin as part of their treatment in relation to genetic variations. This work is summarized in chapter 5.

CHAPTER 2

THE EFFECT OF CHEMOTHERAPY ON COGNITVE FUNCTION: A REVIEW OF CURRENT KNOWLEDGE

Introduction

Over the last 20 years, increasing evidence has suggested that treatments for non-central nervous system (CNS) tumors can have both acute and long-term effects on cognitive functioning. Understanding these cognitive changes and the impact on survivors' functioning is critical, because thousands of patients are treated worldwide each year, and the number of long-term survivors who may have to cope with these cognitive changes is growing (5). Cancer survivors have coined the terms "chemobrain" and "chemofog" to refer to the problems that patients experience with their memory/or concentration during and after completing chemotherapy. For most patients the problem appears subtle, and often improves after ceasing therapy, however for a subset of people the symptoms are sustained and can impact their educational and career decisions, and reduce their quality of life (6). In this review we will discuss the major cognitive domains believed to be affected by chemotherapy, possible mechanisms of impairment, risk factors and possible management strategies.

Affected cognitive domains

Cognitive decline post chemotherapy has been studied previously. Initial studies were mainly cross-sectional in design and focused on breast cancer. Subsequently there have been longitudinal studies in breast cancer as well as other cancer types. Table 1 provides a summary of select studies evaluating cognitive function post chemotherapy.

Ahles et al. (7) evaluated the neuropsychological impact of chemotherapy regimens in long-term (>5 years post diagnosis) survivors of breast cancer and lymphoma. They compared survivors treated with local therapy and survivors who were treated with systemic chemotherapy (Table 1). After controlling for age and education,

they found that survivors who had been treated with systemic chemotherapy scored significantly lower on the battery of neuropsychological tests compared with those treated with local therapy only ($P < .04$), particularly in the domains of verbal memory ($P < .01$) and psychomotor functioning ($P < .03$). Survivors treated with systemic chemotherapy were also more likely to score in the lower quartile on the Neuropsychological Performance Index (39% v 14%, $P < .01$) and to self-report greater problems with working memory on the Squire Memory Self-Rating Questionnaire ($P < .02$). This study however was cross sectional, with no pre-treatment data available. The exact impact of therapy cannot be determined from this study design. Furthermore, patients who received tamoxifen were allowed in the study, recent studies have showed a negative effect of tamoxifen on cognition (8). In addition, there were multiple therapeutic regimens, and more than one type of cancer, confounding the results. Nevertheless, this study is important because it is one of the first studies to support that deficits in cognitive functions are associated with systemic chemotherapy. The overall effect of treatment can be diffuse, and that is expected given the mixture of systemic treatment regimens. It was also one of the first studies to point out that impairments can be long term.

The study by Wefel et al. (9) was one of the first important prospective longitudinal studies with pretreatment data. 18 patients with breast carcinoma underwent a comprehensive neuropsychological evaluation before treatment, 3 or more weeks after the cessation of the administration of drugs that were used to control nausea and emesis or that were known to have CNS activity (approximately 6 months after baseline), and 1 year post chemotherapy. Those patients received FAC: 5-Flurouracil, doxorubicin, and cyclophosphamide. There were no overall mean differences in cognitive function between patients and controls (normative data). Within-subject analysis at the 6 months point showed that 61% had cognitive declines mainly in attention, learning, and processing speed. Approximately 50% of patients who had declines at 6 months demonstrated improvement at one year post chemotherapy, whereas 50% remained

stable. This study is important for many reasons. Without pretreatment data, 46% of patients would not have had detectable cognitive decline as their post treatment data are within normal ranges. Nevertheless, subtle changes in cognition that otherwise might not be detectable can have a great impact on patients quality of life. Wefel et al. also found that 33% of patients exhibited cognitive impairment even before starting treatment.

Ahles et al. studied the effects of age and cognitive reserve (see the section about populations at risk) on cognitive functioning in patients with breast cancer who received adjuvant therapy (10). Their analysis revealed that older patients with lower baseline cognitive reserve as measured by (Wide Range Achievement Test, ed 3 Reading score) who were exposed to chemotherapy had lower performance on processing speed compared with patients not exposed to chemotherapy. In addition, they found a negative effect of tamoxifen on processing speed and verbal memory in the no-chemotherapy group. They also concluded that chemotherapy had a short-term impact on verbal ability. This study is important because of the relatively larger sample size, in addition to the analytic approach used, the use of multiple comparison groups, and for the longitudinal design with pretreatment data. It is also important because it studied the effect of tamoxifen. The results of this study suggest an effect for hormonal therapy on cognition although it was not powered for this reason. Tamoxifen effect on cognition has been studied previously with indication of effects on verbal memory and executive function (8). However, multiple chemotherapeutic regimens were used and again it is hard to isolate the exact effect of each. And finally, this study showed concordance between objective measures of cognition and self report measures as the chemotherapy–exposed group as a whole reported more cognitive symptoms compared to the other two groups.

Yamada et al. (11) evaluated the effects of chemotherapy on cognition in long-term survivors of breast cancer. Women over the age of 65 and at least 50 years of age at the time of cancer treatment and diagnosis, and at least 10 years post cancer treatment were compared to a demographically matched control group. The chemotherapy treated

group demonstrated worse performance on attention, psychomotor speed, executive functioning, and global cognitive functioning. This study is important because it evaluates cognitive function long term, and in an older population, however, it was a cross sectional study, without pretreatment data. Therefore, it is hard to isolate the effect of chemotherapy from the effect of age on cognitive decline. In addition, the sample was comprised of a cohort of a higher intellect and education, which limits the generalizability of the results. Also, it is hard to isolate the effect of co-morbidity, even though participants with unstable medical and neurological conditions were excluded.

Tager et al. (12) tried to control for some of the variables reported in previous studies. They studied 61 post menopausal women with non-metastatic breast cancer at 3 times: before adjuvant therapy, 6 months after treatment, and at a final 6 months follow up. They controlled for menopausal status, and they had a limited no. of chemotherapy regimens in general. Time-by-treatment interaction was significant in the motor domain ($P = 0.007$) with poorer performance in women treated with chemotherapy. For the other domains, scores did not significantly vary over time by group. Tager's study was more focused. Some important points of discussions were identified

1. Women who were not treated with chemotherapy showed improvements on motor tasks compared to the chemotherapy group and this might be due to practice effect (increases on tests scores that happen when an individual retakes the same or similar tests). Women in the chemotherapy group didn't show an enhanced performance, so the apparent "lack of practice effect" can actually be viewed as a deficit that needs to be accounted for.
2. Motor slowing in women treated with chemotherapy could be secondary to peripheral neuropathy rather than an indication of more general declines in cognitive processing. The incident of chemotherapy induced peripheral neuropathy in cancer treatment is increasing in general. In addition, neuropathy is the dose limiting toxicity of paclitaxel (a taxane), which is a common agent used to treat breast cancer. Thus,

decreased motor functioning could be due to decreased motor nerve conduction rather than general declines in executive functions. The authors recommended the use of the Stroop Interference Task, a measure relatively unaffected by peripheral motor slowing (13,14).

It is worthwhile to point here that this study included patients who are using SSRIs, and hormonal therapy which might confound the results.

In their longitudinal study, Jansen et al. (15) studied the cognitive function in 71 breast cancer women pretreatment and at 1 week, and 6 months post chemotherapy. They used the Stroop test as they followed two groups: patients treated with doxorubicin and cyclophosphamide, and patients treated with doxorubicin and cyclophosphamide and a taxane. They found short term significant decreases in visuospatial skill, attention, delayed memory, and motor function, which improved 6 months after therapy. Deficits in motor function were found almost exclusively in women who received a taxane, they concluded that these changes may be the result of peripheral neuropathy and warrant additional investigation. They also found cognitive impairment in 23% of breast cancer patients before treatment. This study is especially important because a limited number of chemotherapy regimens were studied. However there was no control group, nor corrections for multiple testing.

A recent longitudinal study carried out by Phillips and colleagues (16) compared the effects of chemotherapy and radiotherapy (CT) in breast cancer patients versus the effects of radiotherapy alone (RT). They also had non cancer patients as controls (NC). They found that NC group tended to have improved performance over time compared to the CT and RT groups. Interestingly they didn't find any difference between the CT and RT groups in any cognitive domain.

There have been a few meta-analyses (17-21) regarding this topic. Jim et al. (21) reviewed the evidence in breast cancer survivors who were treated with chemotherapy more or equal than 6 months previously from the years between 1937 to 2012. Patients

treated with chemotherapy performed worse than non-cancer controls in verbal ability and worse than patients treated without chemotherapy in visuospatial ability (both $P < .01$). Age, education, time since treatment, and endocrine therapy did not moderate observed cognitive deficits in verbal ability or visuospatial ability (all $P \geq .51$). However the magnitude of all effect sizes was small. The finding that chemotherapy treated patients performed worse on visuospatial ability compared to other patients with cancer but not with individuals without cancer seems counterintuitive, but that might be because they only had one comparison group for the normal people. Stewart et al. (20) also studied patients with breast cancer who were treated with adjuvant chemotherapy. They found small but significant decline in global cognitive functioning, language, short term memory, and spatial abilities, none of the findings however reached clinical significance. Falleti et al. (19) found declines in the domains of language, spatial function, psychomotor function, attention, executive function and memory. Those were small to moderate effect sizes, and were also in breast cancer patients. Furthermore, they found that the magnitude of impairment associated with adjuvant chemotherapy appears to be less when time since chemotherapy increases. Jansen et al. (18) studied several types of cancers including breast cancer. They included 16 studies, nine of which included patients with breast cancer, and the majority assessed cognition during treatment or shortly thereafter. When patients were compared with normative data, statistically significant medium effect sizes were found in the domains of executive function, information processing speed, verbal memory, and visual memory. Patients treated with chemotherapy performed worse in all domains. When patients were compared with healthy matched controls, small, statistically significant effect sizes were found in language and verbal memory, with patients performing worse. When patients treated with chemotherapy were compared with control patients treated with local therapy or with their own baseline scores, no significant differences were observed. Anderson-Hanley et al. (17) examined the neuropsychological effects of cancer treatment, including

chemotherapy, interferon alfa, interleukin-2, radiotherapy, total-brain irradiation, hematopoietic cell transplant, and biologic therapy. Patients were assessed during treatment or shortly after. Statistically significant medium to large effect sizes were found in the domains of verbal memory, executive function, and motor skill when patients were compared to normative data. Comparing patients with controls, statistically significant small to medium effect sizes were found across all domains. Patients performed worse in all comparisons. Significant effects were not present when comparing patients to their own baseline.

From these data we can see that the declines in cognitive function are global and involve many domains including processing speed, executive function, verbal ability, memory and attention. The effect sizes range between small and moderate, with no clear or robust information on the onset, duration of the decline, cancer type, nor the liable chemotherapy regimen. We also don't know which populations are more vulnerable or what is the biological mechanism behind these cognitive changes.

Possible mechanisms for cognitive declines post chemotherapy

Although the brain is given some protection from systemic treatments by the blood brain barrier, it is increasingly recognized that many chemotherapeutic agents affect brain function. The mechanisms by which chemotherapeutic agents affect cognitive function are largely unknown, but there are many proposed direct/ and or indirect mechanisms which we present here. Figure 1 represents a summary of candidate mechanisms for cognitive dysfunction due to cancer and cancer treatment that we discuss below.

Blood Brain Barrier (BBB)

The tight junctions between capillary endothelial cells in the brain and between the epithelial cells in the choroid plexus effectively prevent proteins from entering the

brain in adults and slow the penetration of some smaller molecules as well. This uniquely limited exchange of substances into the brain is referred to as the blood brain barrier. Passive diffusion across the tight cerebral capillaries is very limited, and little vesicular transport takes place. However, there are numerous carrier-mediated and active transport systems in the cerebral capillaries. These move substances out of as well as into the brain, though movement out of the brain is generally freer than movement into it (22). Generally it has been assumed that most chemotherapeutic agents do not cross the BBB except for methotrexate and 5-Fluorouracil (5-FU). However it was found that some agents such as carmustine, also known as BCNU, and paclitaxel cross the BBB although their levels are very low(23)(24), whether these low levels, which are not efficacious in the treatment of tumors, cause deficits in cognitive function is something that is still to be determined (25).

Conventionally cytotoxic drugs are thought to target the proliferating cells such as the glial and endothelial cells; however recent studies indicate that the neurotoxic mechanisms are far more complex. Dietrich and colleagues (26) administered BCNU, cisplatin and cytarabine systemically to mice. This was associated with increased cell death of oligodendrocytes (myelin forming cells) and of neural progenitor cells (the direct ancestor of all differentiated cell types of the CNS) but astrocytes and neurons were less vulnerable. This pattern was seen in the subventrical zone, the hippocampus, and the major white matter tracts, and persisted long term after repetitive drug exposure (26). In a follow up study, Han et al. examined the toxic effects of 5-FU on the CNS. Consistent with their previous studies, clinically relevant doses of 5-FU administered systemically resulted in significant toxicity to non dividing oligodendrocytes and lineage committed progenitor cells in the subventricular zone, the dentate gyrus of the hippocampus, and white matter tracts (27).

One factor that can determine the levels of chemotherapeutic agents in the brain are drug transporters. A common transporter is P-glycoprotein (P-gp) the protein product

of the multi drug resistance 1 gene. P-gp is expressed in the endothelial cells at the BBB and protects the brain by transporting toxic substances, including chemotherapeutic agents, outside of it. For this reason the level and functionality of Pg-p affects the levels of cytotoxic agents in the brain. Genetic polymorphisms in this gene can affect its expression; consequently it can affect the level as well as the functionality of the protein. See Chapter (3) for discussion of Pg-p genetics. It is also likely that genetic polymorphisms in other transporters are important.

Oxidative Stress and DNA damage

Normal DNA is required for the normal functioning of biological systems. Errors occur during DNA replication but the human body is equipped with mechanisms that correct for this damage (ex: Base Excision Repair, Nucleotide Excision Repair, Mismatch Repair, and Double Strand Break Repair) (28). Mutations in repair genes have been linked to diseases such Ataxia Telangectasia, and Xeroderma Pigmintosum. Those two diseases are characterized by significant neurological symptoms including cognitive deficits (29). In addition elderly patients who are diagnosed with mild cognitive impairment have been shown to have higher levels of oxidative damage in peripheral leukocytes and in the brain on autopsy (30)(31). Many chemotherapeutic agents work by damaging DNA such as alkylating agents. And even though they are targeting cancer cells, normal cells are still affected. The presence of efficient repair systems in normal cells will probably offer some protective effect. For this reason, variation in DNA repair efficiency between people would likely cause differences in the susceptibility to suffering cognitive deficits post chemotherapy, as patients with inefficient repair mechanisms will have more difficulty recovering. Thus one would predict that poorer cognitive functioning would be seen in patients with more DNA damage before and after treatment (25). Also, certain DNA repair polymorphisms are associated with increased risk of cancer (32). Consequently, changes in cognitive function following chemotherapy need

to be evaluated within the context of genetic factors that increase the risk of cancer, but also might increase the risk of cognitive function before treatment (25).

Cytokines and the inflammation response

Cytokines are proteins that are released by activated immune cells in response to inflammation, stress, or direct injury to neurons (33). In addition, they have important roles in physiological CNS function, including the modulation of neuronal and glial cell functioning, neuronal repair and the metabolism of dopamine and serotonin, both of which are important neurotransmitters for the normal cognitive function (34). Recently McAfoose et al. presented a model for cytokine role in cognitive functions including learning and memory (35). Because chemotherapy causes injury to normal tissues, the plausibility exists it could induce the release of cytokines (36). There is evidence that standard dose chemotherapy is associated with increases in cytokine levels, specifically, paclitaxel and docetaxel have been associated with increase in the levels of cytokines (37). Cytokines are relatively large and are not likely to be able to cross the BBB, however recent research has shown that there is significant communication between cytokines outside the CNS and cytokines in the brain and the spinal cord, stimulating the release of central cytokines (34). Cytokine release has been associated with sickness behavior. Nonspecific symptoms of sickness behavior include weakness, decreased mobility, malaise, anorexia, inability to concentrate and a decreased ability to learn (36,38). Similar sickness behavior, including cognitive impairments, is seen with chemotherapy (39). Neuronal damage secondary to cytokine exposure can be caused by various mechanisms including excitotoxic glutamate receptor-mediated damage and oxidative stress. Indirectly, reduced appetite might lead to micronutrients deficiency, or sleep might be deregulated (34). Thus we would expect the production of cytokines within the CNS and other neural changes characteristic of sickness to follow the peripheral induction of cytokines by cancer and/or chemotherapy (39).

Hormonal effects

Chemotherapy can lead to temporary or permanent amenorrhea in women, especially in those older than age 40 (40). Amenorrhea generally occurs within 6–12 months of treatment; however, the frequency varies and depends on the type, dose, and duration of the chemotherapy treatment as well as a patients' age (41). The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-30 trial recently reported a comparison of the menstrual activity in patients on one of three adjuvant chemotherapy regimes. Over 5000 patients were randomly assigned to sequential doxorubicin (A) and cyclophosphamide (C) followed by docetaxel (T) (AC-T), concurrent TAC or AT, which varied in duration (24, 12 and 12 weeks, respectively). Most patients were estrogen receptor positive and received endocrine therapy mainly with tamoxifen. The incidence of prolonged amenorrhea defined as amenorrhea at 12 months from starting therapy varied markedly according to type of regimen with a higher incidence in the cyclophosphamide containing arms, 69.8% and 57.7% for AC-T and TAC respectively, and 37.9% for AT. The AT group without tamoxifen had the lowest rate of amenorrhea. In addition some treatments for cancer can lower hormone levels such as tamoxifen. These agents are given in combination with chemotherapy or as single agents (42). There is evidence that these treatments can have a detrimental effect on cognition even without chemotherapy. Estrogen deficiency was associated with cognitive impairments in the verbal memory domain (8). It is worthwhile to notice here that women who become menopausal as a result of chemotherapy experience a more rapid drop in estrogen than they would during natural menopause. Whether the accelerated decrease causes greater impairment in cognitive function is not clear (43).

Secondary changes in cerebrovascular function due to cardiotoxic agents

Many chemotherapy agents are cardiotoxic. For example anthracyclines intercalate with high affinity into DNA and inhibit the action of topoisomerase II, resulting in DNA strand breaks. Cardiac toxicity from this group of drugs is thought to be related to the formation of free radicals created by the enzymatic reduction of a quinone ring or by combining with iron to form a complex that undergoes redox cycling (44). Accumulating evidence from clinical, neuroimaging, and pathological studies indicates a durable link between cardiovascular disease and cognitive impairment; therefore, cognitive changes could be secondary to cardiovascular changes that could influence cerebrovascular function (45).

Anemia

A study of patients with chronic renal failure showed that cognitive decline was more common among patients whose anemia had not been corrected with erythropoietin (46). The literature suggests that low hemoglobin or anemia increases the risk of incident dementia or cognitive decline, although the available studies are few. It can be imagined that low hemoglobin or diagnosed anemia could impact on future cognitive impairment, either directly by reducing blood oxygen levels in the brain over a sustained period of time or possibly by lowering a threshold or reserve capacity such that an otherwise silent cerebrovascular accident such as a small stroke or transient ischemic attack has a greater impact on subsequent cognition (47). Anemia is common in patients with cancer and is a frequent complication of myelosuppressive chemotherapy. The severity of anemia depends on the extent of disease and the intensity of treatment. Repeated cycles of chemotherapy may impair erythropoiesis cumulatively (48).

Genetics of Neural repair and neurotransmission

We have discussed the effect of these topics in chapter (3) and it is likely that they would have an impact on cognitive function post chemotherapy as well.

Several candidate mechanisms for chemotherapy-induced cognitive changes have been proposed, and it is likely that there are several pathways to cognitive decline depending on the treatment regimens and the particular vulnerabilities of the individual. Given that only some individuals seem to experience long-term cognitive changes following chemotherapy, it might be that several interacting mechanisms are necessary to produce changes in biological systems that translate into changes in cognitive ability (25).

Potential confounders

Levels of homocysteine, B12 and folate

Both vitamin B12 and folate (vitamin B9) are involved in a common metabolic pathway supplying essential methyl groups for DNA and protein synthesis. Vitamin B12 acts as a cofactor for methionine synthase that remethylates homocysteine to methionine by using 5-methyltetrahydrofolate as a methyl donor. Deficiency in either folate or vitamin B12 leads to an increase in total serum homocysteine concentrations. Low levels of B12 and folate and high levels of homocysteine have been linked to poorer cognitive function and cognitive decline (49). For example Feng et al. reported deficits in constructional ability using the Block Design Test, processing speed with the Symbol Digit Modality Test and memory using the Rey Auditory Verbal Learning Test with low levels of B12 (or hyperhomocysteinemia) (50). Thus the nutritional status/ and the levels of enzymes across the B12/folate/homocysteine pathway could potentially be of an influence on cognitive status post chemotherapy.

Thyroid function

There is increasing evidence that subclinical hyperthyroidism is associated with cognitive impairment. “Toxic” effects of thyroid hormones on the brain could be mediated by increased brain oxidative stress caused by the mild hyperthyroidism, which promotes reactive oxygen species production (51). Thus it is important to have knowledge of thyroid function status pre and post chemotherapy, to be able to isolate if the effects on cognition are due to chemotherapy truly.

Anxiety, stress, fatigue and depression

It is likely that cancer related fatigue, and the anxiety and stress associated with cancer would influence cognitive function. However, it seems that there is association between perceived cognitive impairment and anxiety and/or depression but not with objective cognitive testing (6).

Cognitive decline is present before chemotherapy

Studies have found that 20% to 30% of patients with breast cancer have lower than expected cognitive performance based on age and education at the pretreatment assessment (52)(53). Ahles et al. found that patients with invasive breast cancer were more likely to be categorized as exhibiting lower than expected cognitive performance based on neuropsychological testing. Interestingly, lower than expected level of performance does not seem to be related to psychological factors (e.g., depression or anxiety), fatigue, or surgical factors (e.g., type or length of general anesthesia) (52). No explanation for this phenomenon currently exists. However it might be that the biology of cancer (e.g., inflammatory response triggering neurotoxic cytokines) may contribute to lower than expected cognitive performance; and/or common risk factors for the development of both breast cancer and mild cognitive changes over years may exist (e.g., poor DNA repair mechanisms) (5,25).

Which populations are at increased risk?

It is likely that a certain population is more at risk of developing cognitive dysfunction post chemotherapy. The presence of one or more of these factors puts patients at risk of experiencing cognitive declines post chemotherapy. Age has long been recognized as a factor in cognitive decline. Aging is associated with a variety of biologic changes including increased DNA damage, oxidative stress, inflammation, cell senescence, and decreased telomere length (5). The risk of neurodegenerative diseases, such as Alzheimer's and dementia, increases with age (54). Thus it is likely that older adults with cancer would be more susceptible to cognitive dysfunction post chemotherapy compared to younger adults. Ahles et al. (5) proposed the reliability theory which states that to support survival, complex biologic systems have developed a high level of redundancy. Therefore, failure of one or more components may not be problematic if other components are available to support a specific pathway. Therefore, aging is determined by the failure rate of systems (loss of redundancy), which is influenced by the initial extent of system redundancy, the systems repair potential, and factors that increase failure rate such as poor health care, lifestyle risk factors, and/or exposure to environmental toxins. Someone with a low failure rate and/or high repair potential will show fewer signs of biologic aging as they age chronologically, whereas someone with a high failure rate and/or low repair potential will age more rapidly. Therefore vulnerability to post treatment cognitive change does not necessarily depend on a given treatment affecting a specific biologic pathway. One patient may be vulnerable to the DNA damaging effects of a chemotherapy regimen, whereas another patient may be vulnerable to the impact on the hormonal milieu of endocrine treatments. This vulnerability may be strongly influenced by the pattern of systems failure before cancer diagnosis (5).

The second factor that could affect cognitive function is cognitive reserve. The idea of reserve against brain damage comes from the repeated observation of individuals

who manage to function clinically in the face of brain pathology. There are two kinds of reserve that have been reported to make independent and interactive contributions to preserving functioning in the face of brain injury: brain reserve and cognitive reserve. According to the brain reserve model, there is some threshold at which clinical deficits will become apparent and those individuals with more brain reserve require more pathology to reach that threshold. Cognitive reserve refers to how flexibly and efficiently one can make use of available brain reserve. Suggested determinants of cognitive reserve include education, IQ, literacy, occupational attainment, engagement in leisure activities, and the integrity of social networks. Those individuals with higher cognitive reserve are thought to be able to accomplish more for any given level of pathology and brain reserve. In terms of the cognitive processes involved, cognitive reserve may operate by allowing for more flexible strategy usage, ability thought to be captured by executive functions tasks. A popular measure for cognitive reserve is Wide Range Achievement Test (WRAT) (55). The effects of age and cognitive reserve on cognitive functioning were studied in patients with breast cancer who received adjuvant treatments (10). The analysis revealed that older patients, who were exposed to chemotherapy, with lower baseline cognitive reserve as measured by WRAT, had lower performance on processing speed compared with patients not exposed to chemotherapy (10).

Another factor could be the menstrual status before therapy. Menstruating females would be at a higher risk for cognitive decline compared to women who are menopausal pre-chemotherapy. Cancer therapy can cause menopause and decreases in estrogen (42). Estrogen deficiency was previously associated with cognitive impairments in the verbal memory domain (8). In addition, factors that increase the risk for cardiovascular disease would also be considered as factors associated with risk for cognitive decline post chemotherapy. The type of chemotherapy and how neurotoxic it is can also be a factor influencing cognitive decline (56).

And lastly genetic predisposition could be a main risk factor. Small et al. studied the effect of Val158Met SNP of COMT on cancer treatment-related cognitive deficits in breast cancer survivors stage 0 to II. COMT-Val carriers performed more poorly on tests of attention, verbal fluency, and motor speed relative to COMT-Met homozygotes in general. Moreover, COMT-Val carriers treated with chemotherapy performed worse on attention on tests compared to healthy controls although not significant after correcting for multiple comparisons (57). In another study carriers of at least one APOE e4 allele scored significantly lower in the visual memory ($p < 0.03$) and the spatial ability ($p < 0.05$) domains compared to non carriers (58).

Possible chemotherapeutic regimens/ their doses

It is still largely unknown which chemotherapeutic agents are more prone to lead to the development of chemobrain and at what doses. Different combination regimens are administered for different cancer types, which makes it difficult to assess the role of each agent separately. The heterogeneity of the study populations, and treatment regimens, combined with smaller sample sizes makes is hard to compare the effects of different drugs. It is likely that the chemotherapy regimen administered affects the rate of cognitive impairment, with some agents having greater neurotoxicity. For example, in breast cancer, the probability of impairment appears to be higher following cyclophophamide, methotrexate and 5-Fluorouracil (CMF) chemotherapy than after anthracycline-containing regimens. This may account for higher incidence of impairment in breast cancer survivors reported in the earlier studies, in which CMF was generally used, than in the later studies in which methotrexate was generally replaced by doxorubicin or epirubicin (6). The exposure to the drug (dose or frequency) is likely an important factor. For example studies completed in women with breast cancer have shown that patients who received high-dose chemotherapy had more cognitive impairment than those who received standard dose chemotherapy or local therapy(7).

Impaired systemic clearance and/or pharmacogenetic modulation of drug pharmacokinetics can lead to a higher drug concentration in patients. Exposure to higher doses or high concentrations of the parent drug and/or its metabolite has been implicated in increasing the risk of developing chemotherapy associated neurotoxicity. In addition, neurotoxicity can be increased as a result of the additive or synergistic effects of multi-agent chemotherapy, and intrathecal administration (59). Some neurotoxic effects are known about many chemotherapeutic agents, but cognitive effects are somewhat less known (36,56,60).

Specific studies need to be designed to determine the role of each agent, and at what doses. Animal studies can be more focused in determining the role of chemotherapeutic agents in cognitive impairment. For example Winocur and colleagues (61) gave normal mice three weekly injections of a combination of methotrexate + 5-Fluorouracil (CHEMO group) or an equal volume of saline (SAL group). The CHEMO group exhibited deficits on cognitive tasks acquired pretreatment (spatial memory, non matching-to-sample learning, and delayed non-matching to sample learning), as well as impaired new learning on two tasks (conditional associative learning, discrimination learning) introduced post treatment.

Possible management strategies

Potentially reversible causes of the difficulties seen (i.e., endocrine or metabolic dysfunction, anemia, fatigue) should be ruled out or treated. Pharmacologic and behavioral strategies can be helpful in reducing the functional disability of treatment-related cognitive dysfunction (62). Methylphenidate (used in the treatment of attention deficit hyperactivity disorder ADHD) has been previously used in HIV patients and patients with brain injuries (62). 10 mg twice daily has also been reported to be useful in the treatment of attention difficulties, processing speed deficits, and fatigue frequently seen in brain tumor patients and has helped to elevate mood as well (63). Therefore, it is

a candidate drug that can be used to treat cognitive problems after chemotherapy. However, in a randomized phase III study of long acting methylphenidate, it was not proven to decrease cancer-related fatigue (64).

Modafinil, another drug used in the treatment of ADHD, in a single dose regimen of 200 mg has been shown to improve attention, psychomotor speed, as well as depression and drowsiness in patients with advanced cancers (65).

Ferguson et al. studied a cognitive behavioral therapy approach (Memory and Attention Adaptation Training; MAAT) in breast cancer patients. MAAT's four cognitive-behavioral components included: (1) Education on memory and attention; (2) self-awareness training; (3) self-regulation emphasizing arousal reduction through relaxation training, activity scheduling and pacing; and (4) cognitive compensatory strategies training. Compensatory strategies included self-instructional training (covert verbal 'self-guidance' during task performance), verbal rehearsal of auditory information, schedule making, external cueing and outlining written material. Each participant received a MAAT workbook with written information about chemotherapy and memory difficulty as well as step-by-step guides on how to practice and apply the compensatory strategies. Improvements in self-report of cognitive function, quality of life and standard neuropsychological test performance were observed at post-treatment, 2-month and 6-month follow-up (66). Erythropoietin (EPO) has a potential neuroprotective effect (67) and its use has been assessed previously (68), however, caution should be observed when using it due to its adverse effects on cancer growth (69).Other potential treatments include cholinesterase inhibitors (used in Alzheimer's disease), Ginkgo biloba herb (used in elderly patients with dementia), non steroidal anti-inflammatory drugs and antioxidants (6).

Future opportunities for the improvement of cognition studies

The literature on cancer and cancer related cognitive dysfunction is relatively new. There is no set criteria for the definition of cognitive decline, thus it is difficult to estimate the exact prevalence since different studies use different thresholds/set points (70). In addition there are numerous tests used to assess cognitive function, with no set battery of core tests that allow for the comparison between studies. We don't know if a short battery is better at assessing the subtle changes compared to long extensive testing. Studies need to be performed to identify tests that are most valid, reliable, sensitive, and specific for detecting short term and persistent chemotherapy induced cognitive impairments (18). There is also disparity between studies in the control groups used (healthy controls, normative data, non-chemotherapy treated patients). And many studies don't correct of multiple testing and practice effect. Furthermore, many of the studies were in breast cancer patients with little emphasis on other types of cancer. And there are multiple reviews about the topic with relatively low number of studies. For these reasons, the Venice workshop recommended that guidelines should be set for testing of cognitive function (70). And the International Cognition and Cancer Task Force recently made some recommendations to harmonize studies of cognitive function in patients with cancer (71). The next step would be the application of these recommendations in a more focused and systematic way.

We now know that there is a subset of chemotherapy treated patients who suffer from cognition deficits post treatment. This could be a mixed effect of disease load as well as therapy. Processing speed, executive functions, verbal ability, attention and memory are shown to be affected, and these have an impact on survivor's quality of life. Despite the increasing literature regarding cognitive function post chemotherapy, it is still new, and many questions require further explaining. For example: when does the deficit begin? What is time course and recovery over time? What are the exact domains? Which

functions recover faster if any? Is memory for information acquired before chemotherapy affected as much as post-treatment learning and memory? What is the best method to identify cognitive decline? What are the exact mechanisms involved? And what is the relative effect of each chemotherapeutic regimen among other risk factors? The answers for these questions require more focused research. For instance there is not enough information about the types of chemotherapy involved in the cognitive dysfunction, and probably larger studies would allow for separating the effects of certain chemotherapy regimens, and other confounders. Furthermore, there has been a lack of a reliable method on either clinical or molecular grounds to detect patients at high risk of developing cognitive dysfunction postchemotherapy or to predict its final outcome. Identification of a genetic or molecular biomarker conferring liability to chemotherapy induced cognitive impairment will enable us to understand possible mechanisms and eventually develop successful interventions for that subset of patients (72). In our research we summarized and studied the genetic influences on cognition in healthy individuals. Understanding the genetic influences on cognition in healthy people is important to understand the pathophysiological changes post chemotherapy. In addition we identified some genetic influences on cognition in patients with lymphoma. Bigger studies are needed to further elucidate this phenomenon and to describe its characteristics.

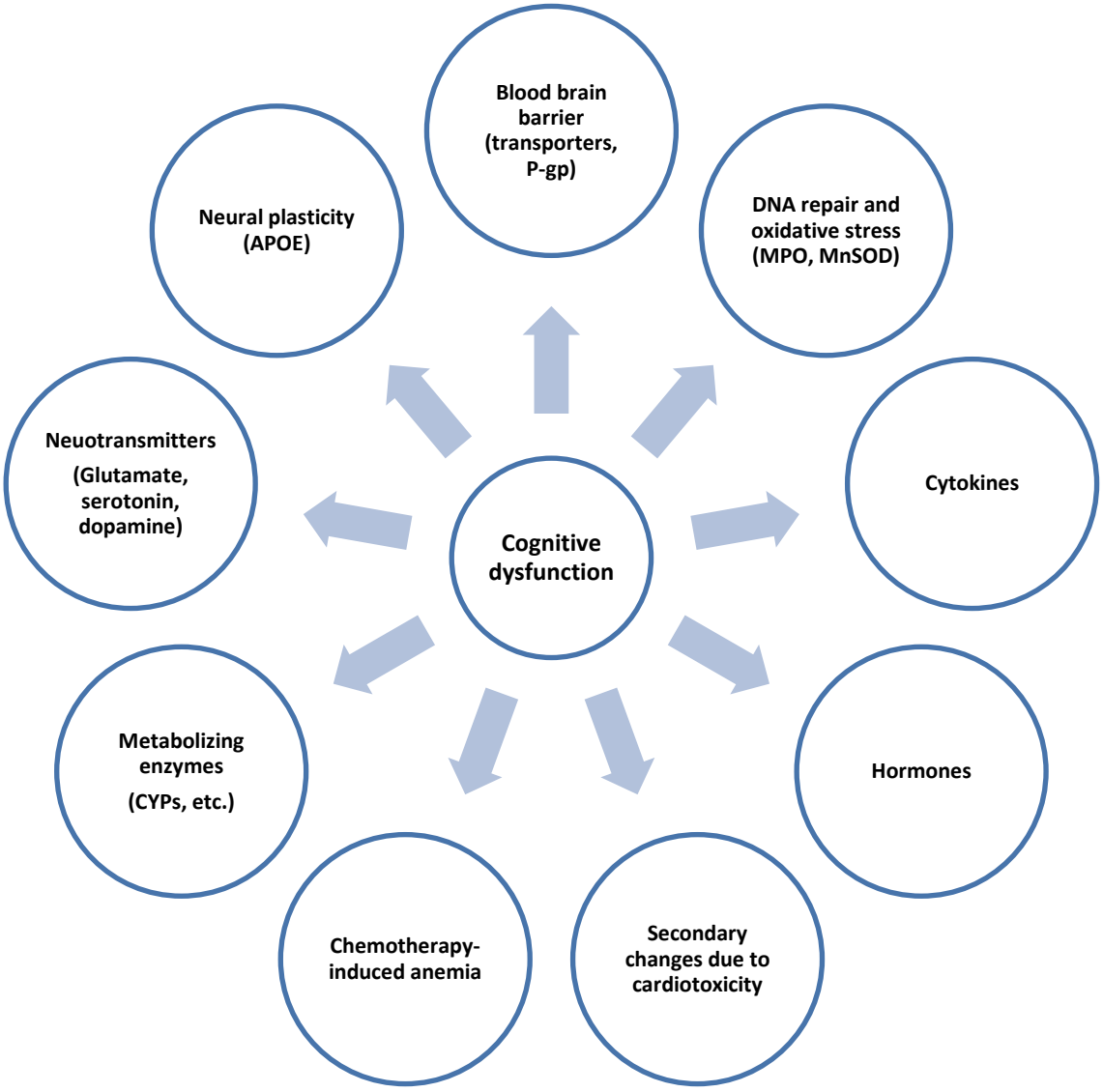

Figure 1 Candidate mechanisms for cognitive dysfunction resulting from cancer and cancer treatment.

Table 1 A summary of some studies of cognitive function post chemotherapy

Study Design	Cancer Type (n)	Chemotherapy regimen	Control group	Results	Reference
Cross sectional 6 years post diagnosis	Breast cancer (Systemic therapy 35+ Local therapy 35) Lymphoma (Systemic therapy 35+ Local therapy 22)	**Breast cancer** (treatment/no) F/M/C 14 F/A/C 14 A/C 3 C/M/F/Vc/P 2 C/Cb 1 **Lymphoma** (treatment/no) C/A/Vc/P 10 Me/Vc/Pr/P 8 Oral C 6 A/B/Vb/Da 3 Me/Vc/A/B/Vb/Da/Pr/P 3 C/A/Vc/E/P 2 C/Vc/Pr/P 2 C/Vc/A/M/Asp/E/Mp 2 A/E/Vb 2 Vb 2 C/A/M/E/Cy/B/Vc/L/P 1 C/B 1 C/E1 C/A/Vc/B/P 1 Me/A/C/Vc/B/P 1	Comparing local therapy (surgery or radiation) and systemic chemotherapy (standard dose chemotherapy)	Lower scores for survivors who had been treated with systemic chemotherapy compared o local therapy on the domains of memory, psychomotor functioning, and self report of working memory.	(7)
Longitudinal Baseline 3 weeks post chemotherapy 1 year post chemotherapy	Breast cancer (18)	F/A/C	Normative data	Decline mainly in attention, processing speed, and learning	(9)

Table 1-continued

Longitudinal Pre-treatment 1 month 6 months 18 months Post chemotherapy	Breast Cancer (123)	A/C/Pac 22 T/A/C 2 F/A/C 1 A/C 18 F/E/C 10 F/M/C 7	Chemotherapy Vs. No Chemotherapy Vs. Healthy controls	Older patients with lower baseline cognitive reserve who were exposed to chemotherapy had lower performance on processing speed compared with patients not exposed to chemotherapy and controls. Chemotherapy had an influence on short term verbal ability.	(10)
Cross Sectional	Breast Cancer (30)	Standard multiagent chemotherapy CMF Or an anthracycline	Demographically matched non cancer control (30)	Worse functioning for cancer patients on global cognitive functioning, attention, psychomotor speed, and executive functioning.	(11)
Longitudinal Pretreatment 6 months after treatment 6 months follow up	Breast Cancer (61)	A/C+D or Pac 14 A/C 7 Ct/M/F 9	Chemotherapy group to a No Chemotherapy group Both with breast cancer diagnosis	Time-by-treatment interaction was significant in the motor domain (P = 0.007) with poorer performance in women treated with chemotherapy.	(12)

Table 1-continued

Prospective longitudinal Pre treatment 1 week after completing the last cycle 1 week and 6 months after the completion of all chemotherapy	Breast Cancer (71)	A/C 22 Or A/C+ Taxane 49	Comparing two chemotherapy groups and to published norms No healthy controls	Cognitive impairment was found in 23% prior to chemotherapy. Significant decreases in visuospatial skill, attention, delayed memory and motor function after treatment followed by improvement at 6 months.	(15)
Longitudinal 6 months and 36 months after completing treatment	Breast Cancer chemotherapy and radiotherapy group (62) Radiotherapy alone (67) Non cancer controls (184)	Radio therapy plus one of the following: A/C 51 A/C/T 13 A/C/Pac 19 C/M/F 8 A/T 2 C/E/F 5 C/E/F/Pac 2	Chemotherapy and radiotherapy to Radiotherapy alone and to No cancer history	Limited support for cognitive dysfunction post chemotherapy. Non cancer controls tended to have improved performance with time on the processing speed Administration of hormonal therapy was not associated with change over time in cognitive performance.	(16)

Abbreviations: Asparaginase, Asp; Bleomycin, B; Carboplatin, Cb; Cyclophosphamide, C; Cytarabine, Cy; Cytoxan, Ct; Dacarbazine, Da; Doxetaxel, D; Doxorubicin, A; Etoposide, E; Fluorouracil, F; Leucovorin, L; Mechlorethamine, Me; Mercaptopurine, Mp; Methotrexate, M; Paclitaxel, Pac; Prednisone, P; Procarbazine, Pr; Taxotere, T; Vinblastine, Vb; Vincristine, Vc

CHAPTER 3

CANDIDATE GENE POLYMORPHISMS INFLUENCING COGNITVE FUNCTION IN NORMAL PEOPLE AND CHEMOTHERAPY-TREATED INDIVIDUALS

Introduction

A growing number of studies are supporting the hypothesis that some cancer patients with no CNS involvement suffer from cognitive deficits after receiving systemic chemotherapy (11)(7)(18)(6)(73). General cognitive ability is known to be highly heritable (74). For this reason genetic susceptibility is likely one of the many variables that affect cognitive changes post treatment (25). Understanding the biological mechanisms underlying cognition in healthy individuals is important in order to understand the pathophysiological changes post chemotherapy. If we can predict the group of people who are more likely to perform poorly in certain cognitive domains, under normal conditions, then we can identify the subset of genetically-susceptible patients who are more likely to suffer from cognitive decline post chemotherapy. Specific management strategies can then be developed for that group. Understanding the gene pathways involved in different cognitive processes will also help in understanding and finding new drug targets for many other diseases such as Alzheimer's disease and schizophrenia (74).

Cognition is a complex trait and is therefore likely to be underpinned by many genes, each with a relatively small effect. To reduce complexity involved, most investigators have attempted to fractionate global cognitive function into discrete cognitive processes via neuropsychological investigations. Performance in these specific domains can then be statistically linked to the functional activity of particular proteins, and to genetic variants or (SNPs) accounting for these functional differences. This is known as an association study and accounts for a major component of research into

complex traits like cognition (75). Genotype-phenotype association doesn't imply causation. Only by placing the association within the appropriate neurobiological background can a plausible mechanistic model be established (76). Most of the information that we have on genes come from studies tailored to certain diseases such as schizophrenia. While this is very important, it often limits the types of genes, or cognitive domains studied. We have a good idea of genes involved in psychiatric diseases, but their relation to actual cognition and molecular mechanisms are largely unknown. And often the instruments used to measure cognition are not standardized, which adds another layer of complexity.

In our genome, there are various types of nucleotide variations (termed polymorphisms: tandem repeat segments (microsatellites, consisting of 2–100 nucleotide repeats, and minisatellites, consisting of 1–20 kilo base pair repeats); deletions; insertions; duplications; and single nucleotide polymorphisms (SNP). SNPs are the most common type of variation, accounting for 90% of known nucleotide variations, and it is estimated that they occur once in every 100–300 base pairs. SNPs can occur in any region of the genome. SNPs in exonic regions can be either non-synonymous, leading to an amino acid change and affecting the protein, or silent polymorphisms, not leading to an amino acid change and not affecting the protein. SNPs in intronic regions can produce changes in the protein sequence if they are located in a splicing site and can alter gene expression if they are located in a region encoding micro RNAs. SNPs in promoter regions can increase, decrease or have no effect on gene expression (77). Polymorphisms can influence brain function through effects with a range of specificities: from effects limited to one receptor type, to effects on neurotransmission systems, to whole brain effects on neural plasticity (74).

Here we provide a review of SNPs in genes that were previously related to cognition, we describe what we know about their effect in normal individuals and what has been done regarding those SNPs in cancer patients.

Single nucleotide polymorphisms that influence cognition in non-cognitively-impaired adults

1- Neuronal Plasticity

APOE

Apolipoprotein E is a polymorphic 299-amino acid protein. The APOE gene resides on chromosome 19 and codes for three alleles: apoE2, apoE3, apoE4. These alleles are a result of two missense single nucleotide polymorphisms (SNPs), rs429358 and rs7412, which result in amino acid changes at residues 112 and 158, respectively. ApoE3 has Cys-112 and Arg158, whereas apoE4 has arginine at both sites, and apoE2 has cysteines. It is worthwhile to note here that ApoeE2 has a defective receptor binding and results in type III hyperlipoproteinemia (78,79). ApoE represents the major lipoprotein within the CNS, where it is mainly synthesized by astrocytes. It has been suggested that one role of apoE in the brain may be an involvement in the mobilization of cholesterol in the CNS, where it is required for neuronal repair and maintenance of synaptodendritic connections. There is increasing evidence that points to an important role for apoE4 in neurodegeneration in general and Alzheimer's disease in particular(79).

In the past 10 years, there have been numerous studies that looked at the effect of ApoE on cognitive function in non cognitively-impaired adults, including two meta-analyses (80,81). Small et al. found statistically significant differences between the E4 and non-E4 groups in global cognitive functioning, episodic memory and executive functioning. In each case the E4 performed more poorly than the non-E4 group. However, the effect sizes were small in each group. They also found that older age and apoE4 heterozygosity was associated with smaller E4- related impairments (80). The results of the meta-analysis done by Wisdom et al. (81) are in line with the one written by Small et al. ApoeE4 carriers performed more poorly than apoE4-non carriers on all of the cognitive domains; significant difference were found on global cognitive functioning,

episodic memory, executive functioning, and a small effect impacting perceptual speed. In contrast to earlier studies, Wisdom et al. analysis revealed that increases in age result in significantly larger differences between apoE4 carriers and non-carriers on measures of global cognitive functioning and episodic memory. These studies indicate that significant apoE4-related deficits do exist although small in magnitude and specific only to some domains of neurocognitive functioning in cognitively healthy adults.

2- Neurotransmitters (Dopamine (COMT), serotonin (5HTTLPR), glutamate (mGLUR3)

Dopamine (Catechol- O- Methyl transferase COMT)

Catechol-O-methyl transferase (COMT) is a gene that is involved in the degradation of dopamine and affects its flux in the prefrontal cortex. Dopamine has been studied as a neurotransmitter for tuning neuronal and circuit responses during cognitive processes (75). The first clues to the involvement of dopamine in cognitive process came from studies of patients of Parkinson's disease (PD). For example L Dopa induced relative increases in blood flow to the right dorsolateral prefrontal cortex and improved high-level cognitive deficits in PD patients (82). However the relationship between dopamine and cognition is not linear. Dopamine does not always enhance cognitive performance and may retard it depending on the basal dopamine level. Thus, an inverted U shaped curve is often used to characterize the relationship between dopamine levels and cognitive performance (83). COMT is the main enzyme responsible for the degradation of dopamine in the prefrontal cortex. Both pharmacological and genetic manipulations of COMT have clarified its role in dopamine metabolism in rodents. Under basal physiological circumstances both COMT inhibition and genetic deficiencies of COMT have little effect on dopamine concentrations in the striatum, where the dopamine transporter and monoamine oxidase (MAO) offer efficient routes for elimination. In contrast, dopamine concentrations were increased in the frontal cortex especially of male

animals deficient in COMT. There were also behavioral and emotional changes in the COMT knockout rodents, and some of these effects were sexually dimorphic (84). COMT is located on the long arm of chromosome 22, and contains a common SNP in exon 4 (Val158Met). This SNP results in an amino acid substitution of Valine (Val) for Methionine (Met). The two different amino acids cause difference in thermo-stability of the enzyme. The Val allele is more stable, and therefore it is associated with greater enzyme activity and hence greater dopamine degradation than the Met allele which has one quarter of the enzyme activity of the Val allele at body temperature (85). And for this reason, Val allele carriers are often expected to have lower cognitive functions, but this might be dependent on the basal level of dopamine in the individual.

Data on COMT Val158Met comes mainly from studies on patients with schizophrenia and their unaffected siblings and healthy controls. In accord with what is expected, most of the studies of this SNP reported higher cognitive performance associated with the Met allele. For example, Egan et al. in an original study associated the Met allele with fewer perseverance errors on the Wisconsin Card Sorting Test (a measure of executive function) and reduced prefrontal function during a working memory task in patients with schizophrenia, their unaffected siblings and healthy controls (86). The Met allele was also associated with better performance in the processing speed, attention in patients with schizophrenia, and episodic and semantic memory in healthy males. Also, carriers of the Val allele compared with carriers of the Met/Met genotype performed worse on executive functioning and visuospatial tasks (87,88). On the other hand, many studies showed no association. Barnett et al. studied the collective evidence regarding COMT val158Met polymorphism and cognitive function in healthy individuals as well as patients. They found a small but significant association between the COMT Met/Met genotype and higher IQ score, which did not differ significantly by sample ancestry, sex distribution, average age of sample, or patient status. However, they didn't find an association between genotype and indices of memory or executive function (89). This

might be due to the limitations of meta-analyses in general, as they are limited by the quality of the studies included in addition to the heterogeneity of those studies. A recent study investigated the interacting effects of other genes in addition to COMT on cognition. Wishart et al. found that COMT genotype predicts executive ability in healthy adults as measured by the Trail-Making Test, even after co-varying for demographics and Apolipoprotein E, brain derived neurotrophic factor and ankyrin repeat and kinase domain containing 1 genotype (ANKK1). There was an interaction between the COMT and ANKK1. People with the Val allele of COMT and the T allele of ANKK1 showed the poorest performance (90). This interaction suggests that genetic variations related to central dopaminergic systems may act together to influence differences in executive abilities.

Delineating the role of COMT Val158Met is not simple and it reflects the complexity of the role of dopamine in cognition. Nevertheless, COMT Val158Met has been and is expected to still be a great research tool to explore this area. We recommend the reviews by Savitz et al. and Dickinson et al. for a deeper discussion of this subject (75,91).

Serotonin: 5HTTLPR (serotonin-transporter-linked polymorphic region)

There is strong evidence that the serotonin (5-HT) system, as well as mechanisms for its facilitation or inhibition, is involved in cognitive processes (92). The human serotonin transporter (5HTT, SLC6A4) is mapped to chromosome 17q11.2 and is organized in 14 exons. The 5HTTLPR is a common 44 base pair deletion in the transcriptional control region upstream of the 5HTT coding sequence. This deletion results in two different alleles. The short allele (S) is the allele containing the deletion, while the long allele (L) is the one which contains the 44 bp segment. The basal activity of the long variant is more than twice higher than that of the short variant, leading to

reduced expression and serotonin uptake with the short allele. It is worthwhile to notice here that there was no difference in expression between the L/S or the S/S genotypes and differences were only seen when compared with the L/L genotype suggesting that the polymorphism has more of a dominant-recessive effect (93,94).

The 5HTTLPR has been studied extensively, and has been related to depression, alcohol abuse, suicidal behavior, as well as personality disorders (95)(96)(97)(98).

The majority of studies found that having the L allele is better (see below), but on the other hand, some studies reported superiority for the S allele.

Evidence for the L allele:

1- In their study, Lesch et al. reported higher neuroticism scores for individuals with one or two copies of the S allele. In addition, individuals with the S allele scored higher on Tension and Harm Avoidance, two measures of anxiety. This result was replicable in their population analysis as well as their family-based associations (94).

2- Welhelm et al. reported fewer problem- solving strategies in response to stress with S allele carriers compared to the L allele, this genotype effect was greater in males. They also proposed that S allele carriers face a gene related propensity for greater emotional reactivity that precludes them from drawing on problem solving strategies for dealing with stress (99).

3- Fiedorowicz et al. reported that heterozygotes for the L_A allele demonstrated significantly higher scores on BRANS (Repeatable Battery for the Assessment of Neuropsychological Status) compared to the L_G and the S alleles, they also demonstrated higher scores on the BRANS subscale for attention, and lower interpersonal sensitivity. $L_{A/G}$ is a polymorphism in the long allele, the L_A being the only high functioning allele (100).

4-Qinghua He et al. studied the effect of 5HTTLPR on decision making under ambiguity and risk in a large Chinese sample. They found that homozygous for the S allele had lower scores than L carriers on the first 40 trials of the Iowa gambling task

(IGT) as a measure of decision making under ambiguity. They also exhibited higher loss aversion than L carriers as measured by the loss aversion task, a measure of decision making under risk. These results were independent of subjects' intellectual or memory abilities, and they were gender specific. Male subjects with L allele had significantly higher scores on the IGT than males who were S allele homozygotes, but only a trend was seen in females, also higher loss aversion was seen for the S homozygotes than L carries for males, but not for females (101).

5-Payton et al. studied the influence of polymorphism in 5HTTLPR on cognitive abilities and cognitive decline. 5HTTLPR was not associated with cognitive decline, but homozygous wild type LL individuals scored significantly higher than heterozygous individuals on delayed recall, a test of memory, while they didn't differ from homozygous mutant SS (102).

In addition to the previous studies, having the S allele was associated with disease. For example Rotondo et al. reported a higher frequency of S allele of 5HTTLPR in bipolar disorders without panic disorder (103), and Vijayan et al. reported higher frequency of the S allele carriers in south Indian patients with schizophrenia (104).

Evidence for the S allele:

1- Volf et al. found that subjects with SS and LS demonstrated higher verbal creativity scores in comparison with the LL genotype. In addition, homozygous individuals scored higher in figural creativity in comparison to LS and LL (105).

2-Borg et al. reported that carries of the S allele had a superior performance on the Wisconsin Card Sorting Test compared to the LL genotype. However there was no association with other cognitive tests including the global IQ measured with Vocabulary and Block Design from the Wechsler Adult Intelligence Scale Revised (WAIS-R), verbal short time and verbal learning assessed with the Claeson-Dahl Memory and Learning Test, Controlled Oral Assessment (106).

Difference in spatial attention:

Carlson et al. found that short allele carriers non-consciously orient spatial attention towards masked fearful faces (107).

However, there were a few negative studies that tried to relate 5HTTLPR to cognition:

1- Schultz et al. studied the effect of 5HTTLPR on late life phobia and cognition. They didn't find a significant association between genotype and phobic anxiety, although there was a trend. However, they detected a significant association between phobic anxiety and cognition (108).

2- Barnett et al. didn't find an effect of 5HTTLPR on cognition in nearly 6000 children. They used the Wechsler Intelligence Scale for Children (WISC) 3rd edition as a measure of IQ, Opposite Worlds task from the Test of Everyday Attention for Children Battery as a measure for verbal inhibition, Count Span task as a measure for working memory (109).

The reasons for inconsistencies regarding 5HTTLPR literature could be that people used different comparison groups (LL vs. LS or SS, LL or LS vs. SS, LL vs. LS vs. SS), although mRNA data indicate no difference in expression between the LS or the SS genotypes and differences were only seen when compared with the LL genotype (93,94). Moreover, the presence of a SNP in the L allele (L_G/L_A) modifies the effect of the L allele. Other reasons are common to all other SNPS and are summarized below.

<u>Glutamate– special focus on metabotropic glutamate receptor 3 (mGLUR3)</u>

Glutamate (GLU) is the most abundant excitatory neurotransmitter in the central nervous system (CNS). It is involved in different neural processes including neuronal development, synaptic plasticity, and neuronal toxicity. It has been associated with learning and memory and believed to have a role in long term potentiation (110,111).

Glutamatergic neurotransmission in the CNS is carried out by two broad types of glutamate receptors: ionotropic and metabotropic. These include the ligand-gated ionotropic GluRs (N-methyl D-aspartate (NMDA), a-amino-3-hyroxy-5-methylisoxazole-4-proprinoic acid (AMPA) and kainate receptors) and the G-protein-coupled metabotropic GluRs (mGluR1-8). The second class of GLU receptors, the metabotropic receptors, can be further grouped into 3 groups based on their primary sequence and signal transduction similarities. Group I contains mGluR1 and mGluR5, Group II contains mGluR2 and mGluR3 whereas Group III contains mGluR4, mGluR6, mGluR7 and mGluR8. mGluRs function as receptors that fine-tune the GLU transmission as they are connected to various intracellular signaling molecules. After its release, the remaining GLU in the cleft is rapidly removed via a family of excitatory amino acid transporters (EAATs) at the presynaptic neurons or glial cells. The removal of the excess GLU at the synaptic cleft prevents excitotoxicity due to prolonged excitatory synaptic transmission which may lead to neuronal death (112). Drugs that target ionotropic glutamate receptors are not considered therapeutically useful because of the ubiquitous involvement of these receptors in mediating fast synaptic transmission throughout the CNS. Metabotropic glutamate receptors on the other hand may provide important pharmacotherapeutic targets for psychiatric disorders associated with increased or decreased glutamatergic neurotransmission. These receptors modulate synaptic neurotransmission, and the heterogeneous localization of at least eight subtypes of mGluRs (mGluR1 to mGluR8) with distinct functional properties suggests that glutamatergic neurotransmission may be modulated in an anatomically and functionally distinct manner. In addition, the group II family of mGluRs, which consists of mGluR2 and mGluR3, is primarily distributed in forebrain regions. Stimulation of this group of mGluRs mediates presynaptic depression and decreases evoked release of glutamate, suggesting that these receptors regulate activated glutamate release by presynaptic mechanisms (113). Mice and rat studies have provided a wealth of information regarding glutamate receptors. For example, these

studies have showed that mGLUR3 has a neuroprotective role in astrocytes, and that activation of mGLUR2 might be harmful in terms of excitotoxicity (114,115). Many studies have reported a role for mGLUR3 in schizophrenia and linked it to cognition through endophenotypes (phenotypes on the pathway between the gene of interest and the complex disease phenotypes). Thus it is likely that variation in the mGLUR3 gene will influence normal cognition in healthy adults.

The human mGLUR3 or GRM3 is located on chromosome 7q21.1-2 and spans 220 kilobases (111). Most of the information regarding this gene comes from studies performed in schizophrenia patients and controls. Fujii et al. studied six SNPs that span the gene and are approximately 50 kb apart (rs274622, rs724226, rs917071, rs1468412, rs1989796, rs1476455). They found differences in allele frequency distribution of the SNP rs1468412 between schizophrenics and controls in a case-control study of a Japanese population(116). Egan et al. followed up on this work and studied some of those SNPs among others on risk for schizophrenia and on intermediate cognitive, physiological and molecular phenotypes related to schizophrenia. The G/G genotype of SNP rs6465084/hcv11245618 had a higher verbal list learning scores than other groups and the G/G genotype had a higher score on verbal fluency compared to the homozygous A/A genotype, in addition, the A allele of rs1468412 was associated with poorer performance on both verbal fluency and verbal list learning, and the C allele of rs2289595 was associated with lower verbal fluency. The A/A genotype of rs6465084/hcv11245618 was also associated with more activation in dorsolateral prefrontal cortex as measured by Functional Magnetic Resonance Neuroimaging in healthy controls, and the A/A genotype was associated with lower levels of the downstream excitatory amino acid transporter 2 (a glutamate transporter) but not with mGLUR3 levels. This SNP (rs6465084) may not be functional per se, but it may be in linkage disequilibrium with a causative SNP (117). The work by Egan et al. is important because they supported the behavioral data with physiological and molecular data thus

providing a potential mechanism of the effects of this SNP. Mossner et al. also studied the SNP rs6465084 in patients with schizophrenia and controls. They found that patients of the AA genotype performed poorly in the digit symbol test, a measure of attention ($p=0.008$) compared to the other genotypes, while healthy controls didn't show any difference between genotypes, also that the letter fluency test and the verbal learning test in schizophrenia patients did not show a significant difference between the genotypes, in contrast to the previous study by Egan et al. (117,118). Although Schwab et al. didn't find a statistical evidence for association, the A allele of rs6465084 was more often present in worse performers of the Stroop color-naming task as compared to the G-allele carriers (119). And recently Bishop et al. found that rs1989796 and rs1476455 were associated with the presence of refractory global symptoms in patients with schizophrenia as measured by the Brief Psychiatric Rating Scale (BPRS) Total scores. Participants with an rs1476455 C/C genotype had significantly higher BPRS scores than A-carriers. Additionally, participants with the rs1989796 C/C genotype had significantly higher BPRS scores than T-carriers (120). Most of the information regarding the mGLUR3 comes from patients with schizophrenia, and cognitive tests used in those studies are centered on schizophrenia pathophysiology, and around SNPs that showed higher frequency in patients. Thus more studies are needed to explain the role of mGLUR3 polymorphisms in healthy individuals in general and post chemotherapy in specific.

3- Oxidative Stress (MPO)

Myeloperoxidase (MPO)

Myeloperoxidase (MPO) is generally known as the glycoprotein present in neutrophils. In the presence of chloride ions and hydrogen peroxide produced by neutrophils during their activation, MPO catalyses the formation of hypochlorous acid, a potent microbicidal agent. This enzymatic system plays an important role in human defense against microorganisms. The oxidative processes implicated in the destruction of

micro-organisms can sometimes be harmful by acting on adjacent host tissues (121). MPO is also regarded as an inflammation enzyme and has been connected to increased rates of coronary artery disease (122). Increased expression of myeloperoxidase was found in some neurodegenerative diseases such as Alzheimer's disease and Multiple Sclerosis (123,124). The majority of immunoreactive material in Alzheimer's disease brain tissue was localized with amyloid plaques and neurons including granule and pyramidal neurons of the hippocampus. In addition myeloperoxidase is both expressed and enzymatically active in the human brain, although the absolute levels are very low in normal brain tissue (124). Twins with major depressive disorder were found to have higher levels of MPO (125). Thus genetic variants in this enzyme are likely to have a role in normal variation in cognitive function.

MPO is located on chromosome 17q23. Two SNPs that were the focus of several studies are of particular interest. Hoy et al. described the polymorphisms G-129A (rs34097845) which was significantly associated with serum MPO concentrations, the A allele being associated with lower levels (121). On the other hand, Hu et al. report no significant correlation exists in MPO 129 locus polymorphism and serum MPO activity (126). The second one is G-463A MPO polymorphism (rs2333227) is a G-to-A substitution in the promoter region of the gene. The more common G allele increases expression of myeloperoxidase, whereas the less common A allele decreases myeloperoxidase expression, apparently by destroying a binding site for the transcription factor SP1 (127).

In a cohort of adults, aged 70–79 years, Pope et al. found that for participants with the MPO G-463 AA genotype, cognitive decline was 1.58 (95% confidence interval: 1.07, 2.35) times more likely than for participants with the AG genotype and 1.96 (95% confidence interval: 1.33, 2.88) times more likely than for those with the GG genotype and thus they concluded that the MPO AA genotype which is associated with decreased production of MPO was a risk factor for cognitive decline (128). This was the only study

performed in healthy individuals to our knowledge. However, in patients with Alzheimer's disease, Reynolds et al. found that that a higher expressing G-463 GG MPO genotype was associated with increased incidence of Alzheimer's disease in females, and decreased incidence in males (129). Reynolds et al. also found that a significantly higher percentage of male patients with Alzheimer's disease carried the MPO A and APOE e4 alleles relative to men carrying neither allele, and that the MPO AA genotype was associated with selective mortality in men, but not in women. They explained this by suggesting that the -463A allele creates an estrogen receptor binding site that may contribute to these gender differences. And in their transfection essays, they showed that estrogen increased MPO A promoter activity by several times and has no significant effect on MPO G promoter activity (130). Thus although the G allele is associated with higher basal expression (127), the A allele is promoted by hormones such as estrogen (130).

We haven't found information regarding the MPO129 SNP and cognition. Schnabel et al. report a new Val/Ala missense variation in MPO (rs28730837) that is related to its concentrations (131). Further studies are needed to explain the role of these SNPs in cognition.

4- Blood brain barrier integrity
(P-glycoprotein (P-gp)/MDR1)

P-glycoprotein (P-gp)/MDR1, a member of the ABC superfamily, is expressed as a result of transcription of the (ABCB1)/MDR1 gene. P-gp is the best characterized protein of the ABC transporter superfamily due in part to its significant role in conferring a multi-drug resistance (MDR) phenotype to cancer cells that have developed resistance to chemotherapy drugs. P-gp/MDR1 is expressed in the apical membrane of many secretory cell types such as kidney, liver, intestine, adrenal gland, and the blood–brain barrier where the normal function involves the excretion of drugs and their metabolites.

Thus, P-gp/MDR1 plays a critical role in drug disposition (132). It is known that the ABCB1 gene is highly polymorphic. The three most frequently occurring SNPs are C1236T in exon 12 (dbSNP: rs1128503), G2677T/A in exon 21 (dbSNP: rs2032582) and C3435T in exon 26 (dbSNP: rs1045642) (133). Association between genotype and mRNA as well as protein expression have been inconclusive. Studies have reported higher mRNA levels for the TT genotype, but also higher levels for the CC genotype of the C3435T. For protein level some studies reported higher protein levels for the CC genotype while many others reported no association (134,135). Studies performed by Fung et al. suggest a molecular mechanism for the effect of the haplotype including the C3435T instead of individual SNPs. Although C3435T doesn't cause an amino acid change, it causes changes in protein folding which are responsible for subtle MDR1 function and structural changes (133). Studies regarding C1236T have reported no association with mRNA levels, and studies regarding the G2677G>T/A were also inconclusive (134,135). ABCB1 haplotypes composed of different SNPs may better represent changes in P-gp function if the function SNPs prove to be of the same haplotype background. However haplotype results have also been inconclusive. These results have to be interpreted with care, as mRNA undergoes post transcriptional modification, and there might be many confounders regarding MDR1 expression such as co-administered drugs, food substances, and unknown endogenous genetic controls (134,135). Other SNPs might influence protein expression levels such as -129T>C in exon 1, the TT genotype being associated with higher expression in the Placenta in a Japanese population. See Leschziner et al. for a detailed review of the controversial literature regarding genetic association of ABCB1 SNPs and haplotypes with P-gp expression, activity, drug response and disease risk (134).

Genetic associations with molecular or clinical phenotypes have largely been inconsistent. As a result no adjustments in drug dosing have been recommended (135). Nevertheless, these differences might show up in differences in cognitive function. P-gp

effluxes many substrates including toxins, steroid hormones, cholesterol, cytokines, and β-amyloid (135). Thus it is very likely to influence cognitive function in normal healthy adults as well as post chemotherapy in cancer patients. Recent research found that P-gp deficiency at the blood–brain barrier increases β-amyloid deposition in an Alzheimer's disease mouse model (136). And in one study, use of P-gp inducers such as rifampin in mild to moderate Alzheimer's disease patients was associated with less dysfunctional behavior at 3 months. This might be due to increased clearance of β-amyloids due to induction of P-gp (137). It is likely that P-gp affects normal cognitive function in some way and studies that characterize the relation between SNPs in the MDR1 and cognition are worthwhile.

Single nucleotide polymorphisms that were studied in relation to cognition post chemotherapy

A genetic predisposition appears to play an important role in cognitive decline post treatment in cancer patients. However, limited studies have tried to focus on the involved genetic components. To our knowledge only two genes were studied: APOE and COMT.

1- APOE

Only one study was focused on exploring the effects of apoE directly. Ahles et al. studied the relationship of apoE genotype to neuropsychological performance in long-term breast cancer and lymphoma survivors treated with standard dose chemotherapy. In this study carriers of at least one apoeE4 allele scored significantly lower in the visual memory ($p < 0.03$) and the spatial ability ($p < 0.05$) domains. However, neither group differed significantly when compared to norms. This study is limited by the fact that it is cross sectional and has a relatively small sample size (58). ApoE4 has been previously associated with poorer performance compared to non-apoE4 carriers. However, in the

two meta-analyses conducted in non-demented older adults, there was no effect on spatial or the visual memory domains; clearly much still needs to be done.

2- COMT

Small et al. studied the effect of Val158Met SNP of COMT on cancer treatment-related cognitive deficits in breast cancer survivors stage 0 to II. COMT-Val carriers performed more poorly on tests of attention, verbal fluency, and motor speed relative to COMT-Met homozygotes in general. Moreover, COMT-Val carriers treated with chemotherapy performed worse on attention on tests compared to healthy controls although not significant after correcting for multiple comparisons(57). Table 2 A summary of studies of SNPs and their effect in relation to cognitive function post chemotherapy

Reasons for inconsistencies in the literature regarding the effect of gene polymorphisms on cognition

There are many reasons for why there are inconsistencies in the literature. Different cognitive tests were used to measure the same cognitive domain, and some of those tests were not specific. Different genes are likely interacting. The genetic backgrounds of individuals are not identical. Some genes might be masking the effect of other genes. In addition, the same gene could have varying effects based on the cognitive domain studied. There might be also differences between genders. Furthermore, there might be environmental effects (social interaction, education).

In the next chapter, we study the effects of multiple genes on cognition in healthy older adults, in an attempt to explore the influence of genes on various cognitive domains.

Table 2 A summary of studies of SNPs and their effect in relation to cognitive function post chemotherapy

Gene	SNP	Cognitive domain affected	Type of cancer and no.	Time post chemotherapy	Type of chemotherapy reported	Sample Size	Reference
APOE	E4,E2,E3	-Visual memory -Spatial ability -Psychomotor functioning	Breast Cancer Stage (0-2) , 51 Lymphoma All stages, 29	Minimum 5 years post diagnosis	Yes	80	(58)
COMT	Val158Met	-Attention -Verbal fluency - Motor speed	Breast Cancer Stage 0-2), 72 Radiotherapy, 58 Healthy controls, 204	6 months following the completion of treatment	Yes	334	(57)

CHAPTER 4

GENETIC VARIATION AND COGNITIVE FUNCTION IN HEALTHY OLDER ADULTS

Introduction

Cognition is often defined as our ability to reason, understand, learn and adapt (138). Cognition is known to be highly heritable (74). Twin studies have reported up to 80% heritability in the elderly (139). Both cognitive ability and its decline with age are influenced by genetic variation that may act independently or via gene-environment interaction (138). However, there is a paucity of research involving the effects of genetic variations on cognitive function in healthy individuals, and most of the studies that have been conducted involved psychiatric samples. We studied the influence of sixteen single nucleotide polymorphisms (SNPs) in six candidate genes on cognition in a group of healthy older adults using a battery of well validated neuropsychological tests. Our hypothesis is that genetic variations in candidate genes will have an influence on cognitive functions in healthy adults. Specifically, genetic variations in genes that affect the levels of the neurotransmitters in the prefrontal cortex, i.e. serotonin (5HTTLPR), dopamine (COMT), and glutamate (GRM3), will influence cognition. In addition, genetic variations that lead to an increase in oxidative stress, and lower levels or functionality of the efflux pump P-gp across the blood brain barrier, and carriers of the E4 allele of Apolipoprotein E will have worse cognitive performance. Myeloperoxidase genotype will be used as the marker for oxidative stress.

Methods

Patients

Older adults were identified through a query of the decision making studies database at the University of Iowa Hospitals and Clinics under the supervision of Dr. N. Denburg. IRB approval (IRB ID #: 200806751) and patient consent were obtained. Neuropsychological testing and a health interview were conducted to rule out outstanding medical and psychiatric conditions that have the potential do adversely impact cognitive functioning.

Genotyping

DNA was extracted from peripheral blood samples using the QiaAmp Maxi kit (Qiagen, Valencia, CA). Target sequence of MDR1, APOE, MPO, GRM3, and COMT genes containing the polymorphic site(s) amplified using standard PCR conditions in which one of the primers is biotinylated. PCR products were visualized by electrophoresis on 2 % agarose gels stained with ethidium bromide prior to genotyping to confirm the correct band size. Genotypes were determined using Pyrosequencing™. Polymerase chain reaction (PCR) and pyrosequencing primers were designed using Pyrosequencing™ Assay Design version 1.01 software (http://www.pyrosequencing.com). Sample preparation of the DNA template prior to pyrosequencing was performed using the Vacuum Prep Tool (Biotage, Uppsala, Sweden). Then, Pyrosequencing was carried out using the PyroGold SNP Reagents (Pyrosequencing AB, Uppsala, Sweden), and the PSQ 96MA instrument with accompanying pyrosequencing software (Biotage, Uppsala, Sweden). SNPs of interest, primer sequences and PCR conditions are described in Table 3. It is worthwhile to notice here that a primer mismatch was created for the MPO463 (rs2333227) to increase assay specificity. Serotonin genotyping was performed according to the method by Heils et al. (93).

Statistical Analysis

Statistical analysis was carried out using JMP statistical software version 10.0.0 (SAS Institute Inc., NC, and USA). Descriptive statistics were defined by mean and standard deviation (SD). Several association models were explored which included genotype based model (for example homozygous A (AA), homozygous B (BB) and heterozygous (AB) or allele based model (for example: two copies of A compared to one copy of (A). All possible combinations were explored in pairs. For all pairwise genotype analysis, t-tests with equal variance were used when there was no evidence of unequal variance based on the results of Levene's test, while t-test with unequal variance was used otherwise. For constructing the 95% confidence intervals (CI), the pooled SD or the group-specific SD was used based on the results of Levene's test. The pooled SD was used whenever there was no evidence of unequal variance, the individual SD was used otherwise. Genotypes with two categories that have less than three subjects in any category were excluded from the analysis. Non-significant results were not reported.

To test for Hardy Weinberg equilibrium (HWE) the Chi square was used. But if any cell had a count of less than 1 (no more than 20% of cells can have less than 5) then the Haldane Exact test was used. Chi Square and Haldane Exact test were used as provided through the R software (R package version 1.4.1 http://CRAN.R-project.org/package=HardyWeinberg) (140). The false discovery rate method, provided through the Q value package from R, was used to adjust for multiple testing, and was set to 0.5 because our study was designed to be exploratory (R package version 1.26.0 http://CRAN.R-project.org/package=qvalue) One method for multiple tests corrections are to change the p-value or the α value directly (family-wise error rate correction). And one of the most conservative multiple tests correction is Bonferroni correction: When you have n tests, use α/n as the threshold for each test. e.g., When you have 10,000 genes to compare, use 5×10^{-6} as the threshold which is too strict. A new type of multiple tests

correction is to estimate what fraction of the ones called positive (i.e., rejected tests) are false positives. This is called the false discovery rate (FDR).

The q value is similar to the well known p-value. It gives each hypothesis test a measure of significance in terms of certain error rate. The p-value of a test measures the minimum false postive rate for the null hypothesis that is incurred when calling that test significant. Likewise, the q-value of a test measures the minimum FDR (i.e. of the rejected tests) that is incurred when calling that test significant. In other words the q-value for a particular test is the smallest FDR for which the test is rejected (i.e., the test result is called positive). The q-value is something like an FDR-corrected version of the p-value. q = 0.5 means that of all the tests that got q-values smaller than that of this test, 50% of them are estimated to be false positives. All the tests with the q-values smaller than a particular FDR are the positives for the FDR (141-143).

Neuropsychological Evaluation

Cognitive testing involved a comprehensive battery of standardized neuropsychological tests, designed to assess premorbid ability, general mental ability, general intellectual ability, attention, psychomotor speed, anterograde memory, working memory, language, visuospatial ability, executive functions, and emotional functioning. The tests and a short description of each are summarized below. Table 4 provides a summary of the domains studies and measures used.

Premorbid ability

The Wide Range Achievement Test-III Reading subtest (WRAT-III) is a measure of premorbid verbal intelligence. It is also a measure of academic achievement. Word reading ability is maintained across the lifespan and tends to be largely unaffected by neurological disease or insult (e.g., primary or secondary dementia). The test evaluates single-word recognition and pronunciation skills (144,145).

General mental ability

Mini-Mental State Examination: The purpose of this test is to screen for mental impairment, particularly in the elderly. The test consists of a variety of items that assess orientation to time and place, attention/concentration, language, constructional ability, and immediate and delayed recall(144,145).

General intellectual ability

Wechsler Abbreviated Scale of Intelligence (WASI) consists of two verbal subtests, which together create a verbal intelligence quotient (VIQ): Vocabulary, a word definition test; and Similarities, a verbal abstraction task. Similarly, the WASI contains two non-verbal (or performance) subtests that together produce a non-verbal (performance) intelligence quotient (PIQ): Block Design, a task of visuoconstructional abilities, requiring the use of 2, 4, or 9 red and white blocks to reproduce designs presented on stimulus cards; and Matrix Reasoning, a test of visual spatial non-verbal reasoning. Utilizing all four subtests, a full scale IQ may be derived (145).

Attention and psychomotor speed

The Digit Symbol subtest of the Wechsler Adult Intelligence Scale (WAIS) is a measure of visual-spatial working memory; in this test, the participant is presented with a key, which contains corresponding numbers (1-9) and symbols. However, the actual test booklet page contains only numbers, and the participant has 90 seconds to insert the appropriate symbol in as many blank boxes as they are able. The score is the number of boxes filled in correctly (145).

Psychomotor speed

The Trail Making Test A (TMT-A) is a task of psychomotor speed. The participant is required to connect consecutively numbered circles from 1 to 25, as quickly and as accurately as possible (144-146).

The Grooved Pegboard Test measures psychomotor speed, fine motor control, and rapid visual- motor coordination. It consists of a small board containing 25 slotted holes angled in different directions. Subjects are required to quickly place key-like pegs into the grid until complete, once through with each hand (145,146).

Working Memory

Working Memory Index (WMI): WMI is calculated using the sum of scaled scores from the WAIS Arithmetic, Digit Span, and Letter Number Sequencing subtests of the WAIS.

Arithmetic: Patient is asked a series of mathematical questions and asked to determine the answer mentally, without pen and paper. Time taken to answer each question is also recorded. Patient is awarded points based on correct response and time taken to respond (145).

The Digit Span subtest of the Wechsler Adult Intelligence Scale (WAIS) is used to assess auditory-verbal attention/concentration. In this test the participant is read strings of digits that must be repeated in a forward manner, which measures attention, and a backward manner which measures concentration (145).

The Letter-Number Sequencing subtest of the WAIS is used to measure auditory-verbal working memory; in this test, the participant is read strings of digits and letters, and must order what they hear in numerical and alphabetical form, prior to repeating the digits and then letters back to the examiner(145).

The Benton Visual Retention Test-Revised (BVRT-R) is designed to assess visual attention/retention, visual memory, visual perception, and visuoconstructive abilities. The stimuli involve 10 designs, with each design containing one or more figures. Each design is exposed for 10 seconds, followed by immediate reproduction from memory by the participants (144).

Anterograde Memory

The Rey Auditory-Verbal Learning Test (AVLT) is designed to assess verbal learning, immediate memory span, new learning, susceptibility to interference and recognition memory. The test consists of five presentations with recall of a 15-word list, followed 30 minutes later with a free recall trial and recognition test in which the 15 target words are intermixed with 15 wrong ones(144).

The Rey-Osterrieth Complex Figure Test-Delay Condition (CFT-D) is designed to measure visual memory and consists of a complex figure that the participant draws with the stimulus visible; following a 30-minute delay, they draw the figure from memory(144).

Language

The Controlled Oral Word Association Test (COWA) is a verbal fluency (word finding) task. The examiner asks the participant to say as many words as s/he can think of that begin with the given letter of the alphabet, excluding proper nouns, numbers, and the same word with a different suffix, over a 60 second duration. This is done three times (i.e., for three letters) (144).

The Boston Naming Test is a measure of confrontation naming, in which the participant is presented with line drawings (e.g., unicorn, compass), and has 20 seconds to correctly name each object (144).

Visuospatial ability

Rey-Osterrieth Complex Figure Test-Copy Condition (CFT-C) consists of a complex figure. The copy performance measures both visual perception and visual construction. Patient is presented with a picture of a geometric complex figure and asked to reproduce it on a piece of paper (without tracing, but with the figure present) as accurately as possible (144,146).

Benton Facial Recognition Test: is a measure of visual perceptual discrimination that requires the matching of identical or near-identical faces (i.e., shadowed). Initially, the patient is shown a photograph on one person's face and asked to pick the same person out of six photos shown below. In later trials, the patient is shown a photograph and asked to identify three photos of the same person out of six below (as the tasks progresses, photos also become more obscure and taken from different angles)(144).

Executive functions

The Trail Making test is a task of conceptual ability and visuomotor tracking. It is composed of two parts, (parts A and B) provide information regarding attention, visual scanning, and speed of eye-hand coordination and information processing. Part B also assesses the ability to alternate between sets of stimuli, an executive function. The test is given in two parts: Trail Making, Part A (TMT-A) involves drawing a line connecting consecutive numbers from 1 to 25. Part B (TMT-B) involves drawing a similar line, connecting alternating numbers (1-13) and letters (A-L) in order (i.e., 1-A-2-B and so on). Scoring involves total time in seconds and number of errors, thus higher scores indicate poorer function (144)(146).

Wisconsin Card Sorting Test: the purpose of this test is to assess the ability to form abstract concepts, to shift and maintain set, and utilize feedback. It is a measure of problem solving and mental flexibility, in which the participant must "break the code" using the examiner's corrective feedback using the words correct or incorrect in a card task. It requires that participants match individual cards taken sequentially from two packs of 64 response cards to one of four sample cards placed in front of the examiner. Each of the key cards contains a specific shape and color. All response cards have designs similar to those on the stimulus cards but vary with color, geometric form, and number. The participant is required to sort the cards to unknown principles (i.e., color, form, number). After ten consecutive correct responses, the sorting principle is changed

without the participant's knowledge. This procedure is continued until all categories have been completed (144).

Intradimensional-extradimensional set shift: it is a computerized instrument in which compound stimuli are presented in two stages (intra- and extradimensional shift) as shapes overlaid with lines on a computer screen. Participants must learn rules through feedback to shift between intra- and extradimensional stages (147).

Iowa Gambling Task: is a task designed to simulate real life decision making. The participants are presented with four decks of cards. They are told that each time they choose a card they will win some game money. Every so often, however, choosing a card causes them to lose some money. The goal of the game is to win as much money as possible. Every card drawn will earn the participant a reward. Occasionally, a card will also have a penalty. The decks differ from each other in the number of trials over which the losses are distributed. Thus, some decks are "bad decks", and other decks are "good decks", because some will lead to losses over the long run, and others will lead to gains (148).

Emotional functioning

Beck Depression Inventory: The purpose of this test is to screen for depression using self-report statements. The patient checks 21 four-choice statements of increasing severity about a particular symptom of depression presented on a single page (144).

Numeracy

Numeracy is a test of how well people understand basic probability and mathematical concepts such as numerical expressions of risks, probabilities, percentages, and frequencies (149).

Results

Demographics

Thirty nine participants were enrolled (nineteen males and twenty females). The age of participants ranged from 69 to 91 years with a mean of 77.8 years. The cohort was highly educated with a mean 16 years of education (range 12-20). The mean IQ was 120.4 with a range of 99 to 141. Demographics are summarized in Table 5.

Genotyping

All SNPs were in Hardy-Weinberg equilibrium. Genotype and allele frequencies are summarized in Table 6

Associations

Serotonin

The SS genotype of the 5HTTLPR was associated with higher IQ scores (n= 8, mean 128.38, 95% CI: 122.13 to 134.62) compared to the LL and LS genotypes (n=31 mean 118.29, 95% CI: 115.12 to 121.24), equal variance t-test P value 0.006, Q value 0.3686. In addition it was associated with higher performance on the vocabulary subset[1] of WAIS which is incorporated in calculating verbal IQ that is incorporated in calculating the full scale IQ (SS n=8, mean 66.25, 95% CI: 61.76 to 70.74 vs. LL or LS n=31, mean 59.74, 95% CI: 57.46 to 62.02 equal variance t-test P value 0.0127, Q value 0.3479). Also the SS genotype was associated with higher score on the Boston Naming Test[2], another language measure, compared to the LL/LS genotypes. The highest difference was between the SS (n=8, mean 19.63, 95% CI: 18.74 to 20.51) and the LL (n= 9, mean

[1] Means of vocabulary subset are converted to t-score (scaled to a mean of 50 , SD of 10)

[2] Boston Naming Test raw scores

17.44, 95% CI:16.12 to 18.78) genotypes (unequal variance t-test P value 0.0073 Q value 0.204). On the other hand the LL genotype of the 5HTTLPR performed better on the Digit Span test[3] (n=9, mean 13.2, 95% CI: 11.42 to 15.02), a subset of WAIS which measures attention/concentration, compared to the LS/SS genotype (n=30, mean 10.67, 95% CI: 9.68 to 11.65, equal variance t-test, p value 0.016, Q value 0.2016), especially on the digit span reverse which measures concentration.

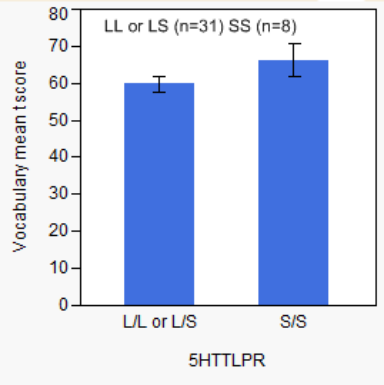

Figure 2 Mean scores on Vocabulary vs. 5HTTLPR genotype

COMT

Association results of the rs4860 were different by domain. Having at least one lower activity allele "Met" was associated with better performance on the Rey-Osterrieth Complex Figure Test-Copy (equal variance t-test P value 0.0403, Q value 0.3892. The mean score for Met/Met or Met/Val was (n=33, mean 33, 95% CI: 31.85 to 34.15) compared to homozygous Val/Val (n=6, mean 29.92, 95% CI: 27.2 to 32.6). We saw the

[3] Means of Digit Span Total score is a t-score (scaled to a mean of 10 of and SD of 3)

same pattern on the Benton Visual Retention Test-Revised. Having at least one Met allele was associated with better performance (unequal variance t-test P value 0.0412, Q value 0.4688. The mean number of correct answers on BVRT for the Met/Met or Met/Val was (n=33, mean 7.27, 95% CI: 6.7 to 7.84) compared to homozygous Val/Val (n=6, mean 6.5, 95% CI: 5.93 to 7.07). And having at least one Val allele was associated with more failures to maintain set on the WCST (n=25, mean 0.72, 95% CI: 0.18 to 1.26) compared to homozygotes Met/Met (n= 7, mean 0, 95% CI: 0 to 0) unequal variance t-test, P value 0.0111 Q value 0.295.

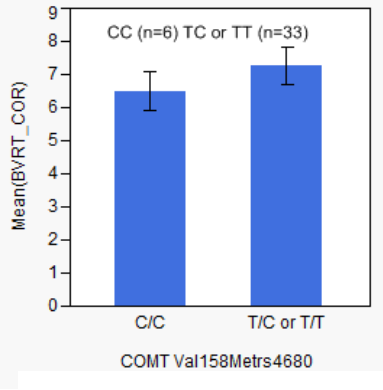

Figure 3 Mean score on Benton Visual Retention Test-Correct answers vs. COMT genotype (C=Val, T= Met)

On the other hand, the higher activity Val allele was associated with better performance on the Rey Auditory Verbal Learning test, Digit Span – Forward subscale of the WAIS, Similarities subscale of the WAIS, and verbal IQ.

Having at least one Val allele was associated with higher scores on the RAVLT[4] (n=31, mean 48.58, 95% CI: 46.08-51.08) compared to homozygotes Met/Met (n=8, mean 41.5, 95% CI: 36.56 to 46.41), equal variance t-test, P value 0.0132, Q value 0.358. Val/Val homozygotes also performed better on the Digit Span Forward test (n=6, mean 11.8, 95% CI: 10.12 to 13.54) compared to Met carriers (n=33, mean 9.73, 95% CI: 8.99 to 10.455) equal variance t-test P value 0.027 Q value 0.163. Val/Val homozygotes also scored higher on the Similarities subscale of the WAIS (n=6, mean 68.17, 95% CI: 63.56 to 72.77) compared to Met Carriers (n=33, mean 60.3, 95% CI: 58.34 to 62.27) equal variance t-test, P value 0.0029, Q value 0.1176. And lastly Val/Val homozygotes had higher verbal IQ scores (n=6, mean 128.5, 95% CI: 120.23 to 136.77) compared to Met carriers (n=33, mean 117.182, 95% CI: 113.66 to 120.71).

MPO

MPO129

The GG genotype of the MPO129 was associated with higher number of errors on stage 8 of the IDED (n=33, mean 12.79, 95%CI: 8.58 to 17) compared to the AG genotype (n=3, mean 4 , 95%CI:-2.57 to 10.57) (unequal variance t-test P value 0.0045, Q value 0.071). The GG genotype also was associated with higher number of errors on the total score of IDED (n=34, mean 24.38, 95%CI: 18.95 to 29.82) compared to the AG genotype (n= 3, mean 10.33, 95%CI: 4.1 to 16.59) (unequal variance t-test P value 0.0001, Q value 0.0063). On the other hand, the GG genotype was associated with higher scores on the Rey Auditory Verbal Learning Test first trial of 15 items (n= 36, mean 5.89, 95% CI: 5.47 to 6.31) compared to the AG genotype (n=3, mean 4.33, 95%CI: 2.87 to 5.8) (equal variance t-test P value 0.0461, Q value 0.2547).

[4] AVLT total words learned from the five trials.

MPO463

The GA genotype of the MPO463 was associated with higher performance on the IGT total score (n=15, mean 21, 95% CI: 6.26 to 35.74) compared to the GG genotype (n= 22, mean -12.5, 95% CI:-30.85 to 5.85) (unequal variance t-test P value 0.005, Q value 0.29, this remained significant after adjusting for age. The GA genotype also scored higher on the total no of words recognized after 30 min delay on the Rey Auditory Verbal Learning test (n=14, mean 29.5, 95% CI: 28.76 to 30.24) compared to the GG genotype (n= 22, mean 28.45, 95% CI: 27.87 to 29.04), equal variance t-test P value 0.0312, Q value 0.198.

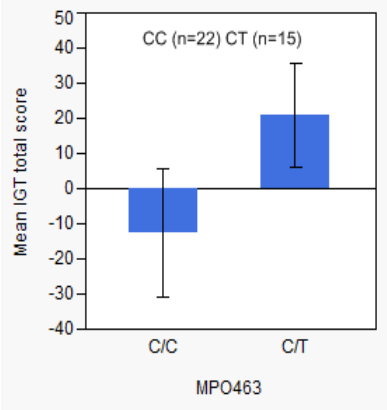

Figure 4 Mean score on Iowa Gambling Task vs. MPO 463 genotype (CC=GG, CT=GA)

The GA genotype was also associated with better performance on measures of IQ. The GA genotype scored higher on the vocabulary[5] subscale of the WAIS (n= 15,

[5] Means of vocabulary subset are converted to t-score (scaled to a mean of 50 , SD of 10)

mean 64.3, 95% CI: 61.05 to 67.6) compared to the GG genotype (n= 22, mean 59.41, 95% CI: 56.7 to 62.12), equal variance t-test, P value 0.0248, Q value 0.4452. In addition they scored higher on the total verbal IQ (n=15, mean 124.47, 95% CI: 119.38 to 129.56) compared to the GG genotype (n=22, mean 116.36, 95% CI: 112.16 to 120.57) equal variance t-test P value 0.0176 Q value 0.2596, and also the AG genotype scored higher on the full scale IQ (n=15, mean ,125.33 95% CI: 120.48 to 129.78) compared to the GG genotype (n=22, mean 118.1 , 95% CI: 114.25 to 121.93) equal variance t-test P value 0.0234 Q value 0.3686. However, carriers of the A allele had a higher average no. of right hand drops on the PEGs test (n= 10, mean 0.8, 95% CI: 0.37 to 1.23) compared the GG homozygotes (n=14, mean 0.142, 95% CI:-0.23 to 0.5) equal variance t-test P value 0.032, Q value 0.21).

GRM3

Hcv11245618/rs6465084

Having at least one G allele of rs6465084 was associated with better performance on the digit span reverse test (n=13, mean 7.62, 95% CI: 6.68 to8.55) compared to the AA genotype (n=26 , mean 6.15, 95% CI:5.5 to 6.8) equal variance t-test P value 0.0138 Q value 0.2173 . Having at least on G allele was also associated with better performance on the total score of digit span (n=13, mean 12.85, 95% CI: 11.36 to 14.33) compared to the AA genotype (n=26, mean 10.46, 95% CI: 9.41 to 11.5) equal variance t-test P value 0.0115 Q value 0.2016. It was also associated with better performance on the WRAT test (n=13, mean 53.7, 95% CI: 51.47 to 55.9) compared to the AA genotype (n= 26, mean 50.65, 95% CI: 49.1 to 52.2) equal variance P value 0.0294 Q value 0.3596. On the other hand having at least one G allele was associated with higher number of left hand drops on the PEGS test (n=11, mean1.18 , 95% CI: 0.7424 to 1.62) compared to the AA genotype (n=13, mean 0.46, 95% CI 0.0573 to 0.8658) equal variance t-test P value 0.0203 Q value 0.1834.

Rs1468412

The AT genotype of the rs1468412 was associated with higher performance the digit span forward (n=11, mean 11.27, 95% CI: 10.02 to 12.52) compared to the AA genotype (n= 27, mean 9.67, 95% CI: 8.87 to 10.46) equal variance t-test P value 0.344, Q value 0.1632. The AT genotype also performed better on digit span reverse (n=11, mean 7.73, 95% CI: 6.7 to 8.75) compared to the AA genotype (n= 27, mean 6.26, 95% CI: 5.6 to 6.9) equal variance t-test P value 0.0195, Q value 0.2289. And on the digit span total scaled scores the AT performed better (n= 11, mean13.1, 95% CI: 11.48 to 14.7) compared to the AA genotype (n= 27, mean 10.63, 95% CI: 9.6 to 11.66) equal variance t-test P value 0.0129, Q value 0.2016. In addition the AT performed better on the R-AVLT total words learned from trials 1 thorough 5 (n= 11, mean 51.64, 95% CI: 47.5 to 55.76) compared to the AA genotype (n= 27, mean 45.7, 95% CI: 43.11 to 48.37) equal variance t-test P value 0.0196, Q value 0.358. On the other hand, the AA had a lower number of left hand drops (n= 14, mean 0.5, 95% CI: 0.11 to 0.89) compared to the AT or TT genotype (n=10, mean 1.2, 95% CI: 0.73 to 1.67) equal variance t-test P value 0.0264, Q value 0.191.

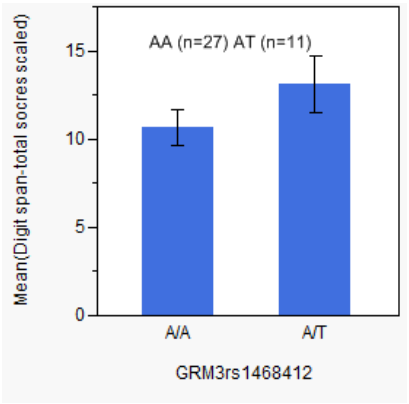

Figure 5 Mean score on Digit Span total score vs. GRM3 rs1468412 genotype

Rs1989796

The association results of this SNP were mixed. The TT genotype had a lower mean right hand time on the PEGs test (n = 7, mean 83.86, 95% CI: 76.06 to 91.66) compared to the CC or CT genotype (n =17, mean 102.118, 95% CI: 87.24 to 116.99), unequal variance t-test P value 0.0275 Q value 0.115. The TT genotype also had a higher score on matrix reasoning subscale of the WAIS [6](n =8, mean 65.25, 95% CI: 62.94 to 67.56) compared to the CT genotype (n =16, mean59.25, 95% CI: 54.43 to 64.07), unequal variance t-test P value 0.0245 Q value 0.2889. And both the TT and CT genotypes (n =24, mean 29.5, 95% CI: 29.17 to 29.9) performed better than the CC genotype on the MMSE (n =15, mean 28.6, 95% CI: 28.13 to 29.07) equal variance t-test P value 0.0028 Q value 0.1638. On the other hand the CC genotype performed better on Numeracy (n =15, mean 9, 95% CI: 8.13 to 9.87) compared to TT genotype (n =8, mean 7.25, 95% CI: 6.05 to 8.44) equal variance P value 0.0227 Q value 0.1598. And lastly, heterozygotes CT (n =15, mean 32.8) performed worse on the IDED total error compared to both the TT (n =8, mean 17.13, P value 0.0231, Q value 0.2426) and the CC genotype (n 14=, mean 16.5, P value 0.0048, Q value 0.1512). But on working memory index with no age adjustment the CT genotype (n=14, mean 103.71) performed better than both the TT (n= 8 , mean 94.5, P value 0.0082, Q value 0.0183) and the CC (n = 15, mean 96.53, P value 0.042, Q value 0.0599).

Rs1476455

The AC genotype performed better on Numeracy (n=8, mean 9.75, 95% CI: 9.01 to 10.49) compared to the CC genotype (n= 30, mean 8.17, 95% CI: 7.38 to 8.96) unequal variance t-test P value 0.0035, Q value 0.1598.

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[6] Score is converted to a t-score (scaled to a mean of 50 of and SD of 10)

Rs 917071

The CT genotype of this SNP was associated with better performance on the BNT[7] (n=15, mean 19.27, 95% CI: 18.73 to 19.8) compared to the CC genotype (n=22, mean, 95% CI 17.63 to 19.1) unequal variance t-test P value 0.0493 , Q value 0.252. The CT genotype was also associated with better performance on the digit span reverse (n=15, mean 7.4, 95% CI: 6.5 to 8.3) compared to the CC genotype (n=24, mean 6.17, 95% CI: 5.46 to 6.87) equal variance t-test P value 0.0338, Q value 0.2366. In addition, the CT genotype of this SNP was associated with better performance on the digit span total score[8] (n=15, mean 12.47, 95% CI: 11.05 to 13.88) compared to the CC genotype (n=24, mean 10.5, 95% CI: 9.38 to 11.62) equal variance t-test P value 0.0338, Q value 0.3348.

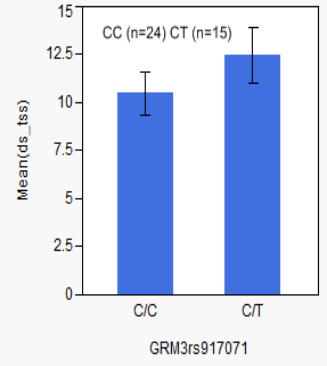

Figure 6 Mean scores on Digit Span total score vs. GRM3 rs917071 genotype

[7] Boston Naming Test raw-scores

[8] Score is converted to a t-score (scaled to a mean of 10 and SD of 3)

And lastly, the CT genotype of this SNP was associated with better performance on the WRAT (n=15, mean 53.47, 95% CI: 51.34 to 55.53) compared to the CC genotype (n=24, mean 50.54, 95% CI: 48.9 to 52.18) equal variance t-test P value 0.305, Q value 0.3596. On the other hand the CC genotype had less no. of left hand drops on the PEGs test (n=11, mean 0.36, 95% CI: -.063 to 0.79) compared to the CT genotype (n=13, mean 1.16, 95% CI: 0.76 to 1.55) equal variance t-test P value 0.0098, Q value 0.118.

Rs274622

The results of this SNP were also different by domain. The CC genotype performed better on IDED total error (n= 3, mean 12.33, 95% CI: -0.17 to 2.837) compared to both the CT or TT genotypes (n=34, mean 24.21, 95% CI: 18.73 to 29.68), unequal variance t-test P value 0.0214, Q value 0.2425). They also performed better on stage 8 of the same test (n=3, mean 6, 95% CI: -.572 to 12.7) compared to CT or TT genotypes (n=33, mean 12.6, 95% CI: 8.35 to 16.86) unequal variance t-test P value 0.0235, Q value 0.2468). Furthermore, they performed better on R-AVLT total words recognized after 30 min delay (n= 3, mean 30, 95% CI: 30 to 30) compared to the TT genotype (n=13, mean 29.08, 95% CI: 28.42 to 29.9) unequal variance t-test P value 0.033, Q value 0.198). And lastly they performed better on the Similarities subset of WAIS [9](n=3, mean 67.33, 95% CI: 60.46 to 74.2) compared to the TT genotype (n=13, mean 59.23, 95% CI: 55.93 to 62.53) equal variance t-test P value 0.0388, Q value 0.4494. On the other hand, the CC genotype performed worse on the Digit Span Forward subtest of WAIS (n= 3, mean 7, 95% CI: 4.65 to 9.35) compared to the CT or TT genotypes (n=36, mean 10.31, 95% CI: 9.63 to 10.99) equal variance t-test P value 0.0095, Q value 0.1035. Also the CC genotype performed worse on the Digit Span Reverse (n=3, mean 6, 95% CI: 6 to 6) compared to the CT or TT genotypes (n=36, mean

[9] Score is converted to a t-score (scaled to a mean of 50 and SD of 10)

6.7, 95% CI: 6.07 to 7.32) unequal variance t-test P value 0.0306, Q value 0.2366. And in the same direction the CC genotype (n=3, mean 8.33, 95% CI: 4.87 to 11.8) performed worse on the Digit Span total score[10] only compared to the TT genotype (n= 13, mean 12.3, 95% CI: 10.65 to 13.97) equal variance t-test P value 0.0435, Q value 0.3426. And lastly the CC genotype performed worse on WMI-Age adjusted (n=3, mean 99.33, 95% CI: 87.18 to 111.49) compared to the CT or TT genotypes (n= 34, mean 113.18, 95% CI: 109.57 to116.79) equal variance t-test P value 0.0333, Q value 0.4314.

Rs724226

The results of this SNP are almost identical to the results from rs274622, in terms of tests and domains, except that this SNP didn't show any significance on the R-AVLT. The AA genotype performed better on IDED total error (n= 3, mean 12.33, 95% CI: -0.17 to 2.837) compared to both the GA or GG genotypes (n=34, mean 24.21, 95% CI: 18.73 to 29.68), unequal variance t-test P value 0.0214, Q value 0.2425). They also performed better on stage 8 of the same test (n=3, mean 6, 95% CI: -.572 to 12.7) compared to GA or GG genotypes (n=33, mean 12.6, 95% CI: 8.35 to 16.86) unequal variance t-test P value 0.0235, Q value 0.2468). And lastly they performed better on the Similarities subset of WAIS[11] (n=3, mean 67.33, 95% CI: 60.46 to 74.2) compared to the GG genotype (n=13, mean 58.693, 95% CI: 55.4 to 61.993) equal variance t-test P value 0.0288, Q value 0.4494. On the other hand, the AA genotype performed worse on the Digit Span Forward subtest of WAIS (n= 3, mean 7, 95% CI: 4.65 to 9.35) compared to the GA or GG genotypes (n=36, mean 10.31, 95% CI: 9.63 to 10.99) equal variance t-test P value 0.0095, Q value 0.1035. Also the AA genotype performed worse on the Digit Span Reverse (n=3, mean 6, 95% CI: 6 to 6) compared to the GA or GG genotypes

[10] Score is converted to a t-score (scaled to a mean of 10 and SD of 3)

[11] Score is converted to a t-score (scaled to a mean of 50 and SD of 10)

(n=36, mean 6.7, 95% CI: 6.07 to 7.32) unequal variance t-test P value 0.0306, Q value 0.2366. And in the same direction the AA genotype (n=3, mean 13, 95% CI: 10.52 to 15.48) performed worse on the Digit Span total score[12] only compared to the GA or GG genotypes (n= 36, mean 17, 95% CI: 15.88 to 18.12) equal variance t-test P value 0.0016, Q value 0.0441. And lastly the AA genotype performed worse on WMI-Age adjusted (n=3, mean 99.33, 95% CI: 87.18 to 111.49) compared to the GA or GG genotypes (n= 34, mean 113.18, 95% CI: 109.57 to116.79) equal variance t-test P value 0.0333, Q value 0.4314.

APOE

The E2 was associated with better performance. Specifically, carriers of the E2 allele, in addition to either the E3 or E4 allele performed better on the ID-ED stage 8 errors (n= 7, mean 1.86, 95% CI: 1.03 to 2.69) compared to the homozygotes E3/E3 allele (n=24, mean 14.79, 95% CI: 9.96 to 19.63) unequal variance t-test , P value 0.00001, Q value 0.000273. In addition, carriers of the E2 allele, as well as either the E3 or E4 allele performed better on the ID-ED total errors (n= 7, mean 12.57, 95% CI: 1.16 to 23.98) compared to the homozygotes E3/E3 allele (n=25, mean 27.12, 95% CI: 21.08 to 33.16) unequal variance t-test , P value 0.0285, Q value 0.2527. The same pattern followed on the Trail making B test, having one E2 allele was associated with better performance (n= 7, mean 0, 95% CI: 0 to 0) compared to homozygotes E3/E3 (n=26 , mean 26, 95% CI: 0.103 to 0.666), unequal variance t-test, P value 0.0094, Q value 0.0928. Moreover, E2 carriers performed better on the PEGS, matrix reasoning, Numeracy. On PEGS, E2 carriers had a lower no. of right hand drops (n=4 , mean 0, 95% CI:0 to 0) compared to the E3/E3 genotype (n= 18, mean 0.44, 95% CI:0.09 to 0.79), unequal variance t-test P value 0.016, Q value 0.1976. Furthermore E2 carriers performed

[12] Raw score, unlike rs274622

better on matrix reasoning subtest of WAIS (n=7, mean 67.43, 95% CI: 65.11 to 69.75) compared to the E3/E3 genotype (n= 27, mean 61.04, 95% CI: 57.1 to 64.97), unequal variance t-test P value 0.0053, Q value 0.104. And they also performed better on Numeracy (n= 7, mean 9.4, 95% CI: 8.9 to 9.9) compared to the E3/E3 genotype (n=26, mean 8.2, 95% CI: 7.3 to 9.1), unequal variance t-test P value 0.0216, Q value 0.1598.

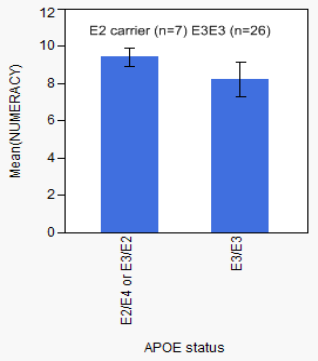

Figure 7 Mean score on Numeracy vs. APOE genotype

In addition the E2/E3 genotype (n=5, mean 19.4, 95% CI: 18.34 to 20.46) performed better than the E4/E3 genotype (n=5, mean 17.6, 95% CI: 16.54 to 18.66) on BNT, equal variance t-test, P value 0.024, Q value 0.2225. And in the same direction the E2/E3 genotype (n=6, mean 53.5, 95% CI: 51.25 to 55.9) performed better than the E4/E3 genotype (n= 5, mean 50, 95% CI: 47.53 to 52.47) on WRAT, equal variance t-test, P value 0.0419, Q value 0.3596.

On the other hand having the E3/E4 or E2/E4 genotype (n=6, mean 6.8, 95% CI: 4.31 to 9.35) seemed to perform better on the WCST-PE compared to the E3/E3 genotype(n=26, mean 12.65, 95% CI: 8.41 to 16.9) unequal variance t-test P value

0.0161, Q value 0.4655, and in the same direction having the E3/E4 genotype (n=5, mean 8.4, 95% CI:7.08 to 9.72) seemed to perform better than the E3/E3 genotype (n=27, mean 6.93, 95% CI: 6.36 to 7.5) on BVRT-correct, equal variance t-test P value 0.0453, Q value 0.4687.

P-gp

C1236T

The TT genotype performed better on the Rey-Osterrieth Complex Figure Copy (n= 13, mean 34.31, 95% CI: 32.5 to 36.11) compared to the CT or CC genotype (n= 26, mean 31.63, 95% CI: 30.36 to 32.9), equal variance t-test P value 0.0191, Q value 0.3892.

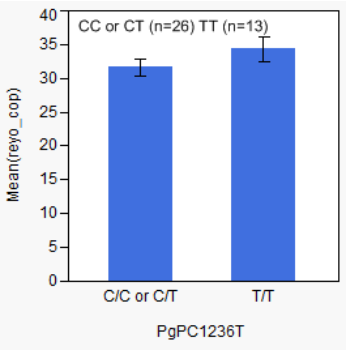

Figure 8 Mean score on Rey Osterrieth Complex Figure-Copy vs. Pgp C1236T genotype

The TT genotype also performed better on Numeracy (n= 13, mean 8.19, 95% CI: 8.9 to 9.99) compared to the CT genotype (n = 16, mean 8.19, 95% CI: 7.1 to 9.28),

unequal variance t-test, P value 0.0348, Q value 0.2143. The TT or CT performed better on Rey-Auditory Verbal Learning test[13] (n= 29, mean 6, 95% CI: 5.47 to 6.53) compared to the CC (n= 10, mean 5.1, 95% CI: 4.57 to 5.63) unequal variance t-test, P value 0.0147, Q value 0.1312. In addition the T allele carriers performed better on Matrix Reasoning Subset of WAIS "scaled score mean 50 SD 10" (n= 29, mean 64.34, 95% CI: 61.12 to 67.57) compared to the CC genotype (n= 10, mean 57.9, 95% CI: 52.4 to 63.4), equal variance t-test, P value 0.0477, Q value 0.4019.

On the other hand, the CC genotype (n= 10, mean 29.7, 95% CI: 29.35 to 30.05) had a higher no. of words recognized after a 30 min delay on R-AVLT compared to the CT or TT genotypes (n= 28, mean 28.64, 95% CI: 28.04 to 29.24) unequal variance t-test P value 0.0028, Q value 0.084. And lastly carriers of the C allele (n= 26, mean 29.42, 95% CI: 29.05 to 29.8) had a higher mean score on the MMSE compared to the TT genotype (n= 13, mean 28.69, 95% CI: 28.16 to 29.22), equal variance t-test P value 0.0289, Q value 0.4057.

G2677A/T

The GA genotype performed better on the IGT-total scores (n=3, mean 40.67 , 95% CI:3.05 to 78.29) compared to the GG (n=8, mean -2,95% CI: -31.55 to 27.55, unequal variance t-test P value 0.0221, Q value 0.4261), and the GT (n= 18, mean 6.83, 95% CI: -14.71 to 28.38, unequal variance t-test P value 0.0325, Q value 0.4699), and also the GA (n=3, mean 40.67, 95% CI: -6.71 to 88.04) performed better than the TT genotypes (n=10, mean -15, 95% CI: -41.85 to 10.05, equal variance t-test P value 0.0417, Q value 0.4824).

The GA genotype also performed better on the Rey-Osterrieth Complex Figure test- Copy (n=3, mean 36, 95% CI: 32.59 to 39.41) compared to the GG (n= 8, mean

[13] First learning trial of 15 items

31.75, 95% CI: 29.66 to 33.84, equal variance t-test P value 0.0398, Q value 0.3892). The GA also performed better (n=3, mean 36, 95% CI: 31.7 to 40.3) than the GT genotype (n= 18, mean 31.31, 95% CI: 29.55 to 33.1, equal variance t-test P value 0.0478, Q value 0.3892). And the TT genotype performed better on the same test (n=10, mean 34.3, 95% CI: 32.16 to 36.44) compared to the GT genotype (n= 18, mean 31.31, 95% CI: 29.71 to 32.9, equal variance t-test P value 0.0296, Q value 0.3892).

The GA genotype performed better on the R-AVLT [14] (n= 3, mean 6.67, 95% CI: 5.6 to 7.69) compared to the GG genotype (n= 8, mean 4.88, 95% CI: 4.25 to 5.5) , equal variance t-test P value 0.0082, Q value 0.1098. And the GT genotype (n= 18, mean 6.11, 95% CI: 5.48 to 6.7) performed better than the GG genotype (n= 8, mean 4.88, 95% CI: 4.33 to 5.41) on the same test equal variance t-test P value 0.0032, Q value 0.0857. On the other hand the GT genotype performed worse on the R-AVLT "total words recognized after a 30 min delay" (n=18, mean 28.5, 95% CI: 27.68 to 29.32) compared to the GG genotype (n= 8, mean 29.75, 95% CI: 29.36 to 30.12, unequal variance t-test P value 0.0073, Q value 0.091.

The GT also performed better on Matrix Reasoning[15] subset of WAIS (n= 18, mean 64.5, 95% CI: 60.3 to 68.7) compared to the GG genotype (n= 8, mean 55.63, 95% CI: 49.32 to 61.93), unequal variance t-test P value 0.0235, Q value 0.289. And lastly, the TT genotype performed better on Numeracy (n=10, mean 9.4, 95% CI: 8.8 to 10) compared to GA, GG, or GT genotypes (n= 28, mean 8.18, 95% CI: 7.3 to 9.04), unequal variance t-test, P value 0.0193, Q value 0.1598.

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[14] First learning trial of 15 items

[15] Score is converted to a t-score (scaled to a mean of 50 and SD of 10)

C3435T

Carriers of the T allele (n= 31, mean 100.74, 95% CI: 97.73 to 103.76) performed better on the WMI[16] compared to the CC genotype (n= 6, mean 88.83, 95% CI: 81.98 to 95.69), equal variance t-test P value 0.0027, Q value 0.01. On the other hand the CC genotype performed better on the R-AVLT average no. of words recognized after 30 min delay (n= 7 , mean 29.7, 95% CI: 29.26 to 30.17) compared to the CT or TT genotypes (n=31, mean28.74, 95% CI: 28.19 to 29.29), unequal variance P value 0.0057, Q value 0.091.

Discussion

Serotonin

There is strong evidence that the serotonin (5-HT) system, as well as mechanisms for its facilitation or inhibition, is involved in cognitive processes (92). The human serotonin transporter (5HTT, SLC6A4) is mapped to chromosome 17q11.2 and is organized in 14 exons. The 5HTTLPR is a common 44 base pair deletion in the transcriptional control region upstream of the 5HTT coding sequence. This deletion results in two different alleles. The short allele (S) is the allele containing the deletion, while the long allele (L) is the one which contains the 44 bp segment. The basal activity of the long variant is more than twice higher than that of the short variant, leading to reduced expression and serotonin uptake with the short allele. It is worthwhile to notice here that there was no difference in expression between the LS or the SS genotypes and differences were only seen when compared with the LL genotype suggesting that the polymorphism has more of a dominant-recessive effect (93,94). In our study, the SS genotype of 5HTTLPR was found to be associated with higher IQ and better performance

[16] No age adjustment

on tests of verbal IQ and language. The SS genotype was associated with reduced reuptake of serotonin, which implies that higher availability of serotonin entails advantages in terms of language. Previously, Volf et al. found that subjects with SS and LS demonstrated higher verbal creativity scores in comparison with the LL genotype. In addition, homozygous individuals scored higher in figural creativity in comparison to LS and LL. (105). Also Borg et al. reported that carries of the S allele had a superior performance on the Wisconsin Card Sorting Test compared to the LL genotype. However there was no association with other cognitive tests including the global IQ measured with Vocabulary and Block Design from the Wechsler Adult Intelligence Scale Revised (WAIS-R), verbal short time and verbal learning assessed with the Claeson-Dahl Memory and Learning Test, Controlled Oral Assessment (106). This emphasizes the need for studies of larger size to explain the discrepancies in the literature. Subjects with the LL genotype of the 5HTTLPR performed better on measures of attention. This is also in line with previous studies. Fiedorowicz et al. reported that heterozygotes for the L_A allele demonstrated significantly higher scores on BRANS (Repeatable Battery for the Assessment of Neuropsychological Status) compared to the L_G and the S alleles, they also demonstrated higher scores on the BRANS subscale for attention, and lower interpersonal sensitivity. $L_{A/G}$ is a polymorphism in the long allele, the L_A being the only high functioning allele (100).

COMT

COMT is the main enzyme responsible for the degradation of dopamine in the prefrontal cortex (84). It is located on the long arm of chromosome 22, and contains a common SNP in exon 4 (Val158Met). This SNP results in an amino acid substitution of Valine (Val) for Methionine (Met). The two different amino acids cause difference in thermo-stability of the enzyme. The Val allele is more stable, and therefore it is associated with greater enzyme activity and hence greater dopamine degradation than the

Met allele which has one quarter of the enzyme activity of the Val allele at body temperature (85). And for this reason, Val allele carriers are often expected to have lower cognitive functions, but this might be dependent on the basal level of dopamine in the individual (83). The association results of the Val158Met rs4860 did not show an inclination to a certain allele. For example, having at least one lower activity allele (Met) was associated with better performance on the Rey-Osterrieth Complex Figure Test-Copy, Benton Visual Retention Test-Revised, and the WCST. This is in line with some of the published literature: de Frias et al. found that carriers of the Val allele compared with carriers of the Met/Met genotype performed worse on executive functioning and visuospatial tasks (88).

On the other hand, the higher activity Val allele was associated with better performance on the Rey Auditory Verbal Learning test, Digit Span – Forward subscale of the WAIS, Similarities subscale of the WAIS, and verbal IQ. These results regarding the Val allele might be counterintuitive initially, but the relationship between dopamine and cognition is not linear (83). Dopamine does not always enhance cognitive performance and may retard it depending on the basal dopamine level. Thus, an inverted U shaped curve is often used to characterize the relationship between dopamine levels and cognitive performance (83). In addition, different behaviors implicate separate brain systems and the optimal range of neurotransmission varies from system to system, for this reason a single curve is insufficient to predict performance: some tasks benefit from extra dopamine “Task B”, while performance on other tasks is disrupted by extra dopamine “Task A” see Figure 9 which is modified from cools et al(83). Moreover, these results may not be applicable to the general population as our sample was composed of mainly high IQ individuals (Range 99-141, Mean 120.4, SD +-9.5).

MPO

MPO129

The GG genotype of the MPO129 was associated with poorer performance on the ID-ED one measure of executive function; on the other hand the GG genotype was associated with higher scores on the Rey Auditory Verbal Learning Test a measure of anterograde memory. Hoy et al. described this polymorphisms G-129A (rs34097845) which was significantly associated with serum MPO concentrations, the A allele being associated with lower levels (121). On the other hand, Hu et al. report no significant correlation exists in MPO 129 locus polymorphism and serum MPO activity (126). We couldn't find any studies that correlate this MPO genotype with cognition, and clearly more work is need to clarify the effect of the SNP on enzyme expression and cognitive functions.

MPO463

The AG genotype of the MPO463 was associated with higher performance on the IGT a measure of executive function compared to the GG genotype. It was also associated with better performance on a test of memory "Total no of words recognized after 30 min delay on the Rey Auditory Verbal Learning". And better performance on measures of IQ. The GA genotype scored higher on the vocabulary subscale of the WAIS, verbal IQ and full scale IQ. However, carriers of the A allele had a higher average no. of right hand drops on the PEGs test. The more common G allele was shown to increase expression of myeloperoxidase, whereas the less common A allele decreased myeloperoxidase expression, apparently by destroying a binding site for the transcription factor SP1 (127). From these data it seems that lower expression of MPO is associated with better performance. However in previous studies, Pope et al. found that for participants with the MPO G-463 AA genotype, cognitive decline was 1.58 (95% CI: 1.07 to 2.35) times more likely than for participants with the AG genotype and 1.96 (95%

CI: 1.33 to 2.88) times more likely than for those with the GG genotype and thus they concluded that the MPO AA genotype which is associated with decreased production of MPO was a risk factor for cognitive decline (128). This doesn't not negate our results, as Pope et al. measured cognitive decline, a change in function over time, and they used the 3MS measure which is a widely used measure of global cognitive function and not specific to any domain. This was the only study performed in healthy individuals to our knowledge. However, in patients with Alzheimer's disease, Reynolds et al. found that that a higher expressing G-463 GG MPO genotype was associated with increased incidence of Alzheimer's disease in females, and decreased incidence in males (129). Reynolds et al. also found that a significantly higher percentage of male patients with AD carried the MPO A and APOE e4 alleles relative to men carrying neither allele, and that the MPO AA genotype was associated with selective mortality in men, but not in women. They explained this by suggesting that the -463A allele creates an estrogen receptor binding site that may contribute to these gender differences. And in their transfection essays, they showed that estrogen increased MPO A promoter activity by several times and has no significant effect on MPO G promoter activity (130). Thus although the G allele is associated with higher basal expression (127), the A allele is promoted by hormones such as estrogen (130). There was no significant difference in genotype frequencies between males and females in our cohort and the two individuals who carried the AA genotype in our set were males. Literature regarding the MPO role in cognition is scarce and more focused analysis is needed.

GRM3

The human mGLUR3 or GRM3 is located on chromosome 7q21.1-2 and spans 220 kilobases (111). We studied 7 SNPs that span this gene (rs274622, rs724226, rs917071, rs1468412, rs1989796, rs1476455, and Hcv11245618/rs6465084).

Hcv11245618/rs6465084

Having at least one G allele of rs6465084 was associated with better performance on the Digit Span-Reverse test, Total Score of the Digit Span, and on the WRAT test compared to the AA genotype. Previous studies found that the G/G genotype of SNP rs6465084/hcv11245618 had a higher verbal list learning scores than other groups and the G/G genotype had a higher score on verbal fluency compared to the homozygous A/A genotype. The A/A genotype of rs6465084/hcv11245618 was also associated with more activation in dorsolateral prefrontal cortex as measured by Functional Magnetic Resonance Neuroimaging in healthy controls, and it was associated with lower levels of the downstream excitatory amino acid transporter 2 (a glutamate transporter) but not with mGLUR3 levels(117). In patients with schizophrenia the AA genotype performed poorly in the digit symbol test, a measure of attention ($p=0.008$) compared to the other genotypes, while healthy controls didn't show any difference between genotypes. Also schizophrenia patients did not show a significant difference between the genotypes on the letter fluency test and the verbal learning test, in contrast to the previous study by Egan et al. (117,118). Our results don't disagree with what previously published as different tests were used. The results do indicate however a superior role for the GG genotype in certain domains. On the other hand having at least one G allele was associated with higher number of left hand drops (worse function) on the PEGS, a measure of psychomotor speed, compared to the AA genotype.

rs1468412

The AT genotype of the rs1468412 was associated with higher performance on the Digit Span Forward, Reverse and total scores compared to the AA genotype. In addition the AT performed better than the AA on the R-AVLT (total words learned from the 5 trials. Previously the A allele of rs1468412 was associated with poorer performance on both verbal fluency and verbal list learning (117). The results of our study are similar

to what was previously found in terms of verbal list learning, but not verbal fluency. On the other hand, the AA performed better than the AT or TT genotype on the number of left hand drops, which points towards a different role for this SNP and for rs6465084 on psychomotor speed, as the results from these two SNPs indicate an opposite role to what is expected.

Rs1989796 + rs1476455

The association results of the rs1989796 SNP were mixed. The TT genotype performed better on right hand time on the PEGs test compared to the CC or CT genotype. The TT genotype also had a higher score on matrix reasoning subscale of the WAIS compared to the CT genotype. And both the TT and CT genotypes performed better than the CC genotype on MMSE. On the other hand the CC genotype performed better on Numeracy compared to TT genotype. And lastly, CT heterozygotes performed worse on the IDED total error compared to both the TT and the CC genotype, but performed better than the TT and the CC on working memory index with no age adjustment. The AC genotype of rs1476455 performed better on Numeracy compared to the CC genotype.

The only information we know about the above two SNPs come from patients with SZ. Bishop et al. found that rs1989796 and rs1476455 were associated with the presence of refractory global symptoms in patients with schizophrenia as measured by the Brief Psychiatric Rating Scale (BPRS) Total scores. Participants with an rs1476455 C/C genotype had significantly higher BPRS scores than A-carriers. Additionally, participants with the rs1989796 C/C genotype had significantly higher BPRS scores than T-carriers (120). Our results fall in line with these results, and also add to what is known.

Rs917071, rs274622 , and rs724226

Rs917071: The CT genotype of this SNP was associated with better performance on the BNT, Digit Span-Reverse, Digit Span total score, and WRAT compared to the CC

genotype. On the other hand the CC genotype had less no. of left hand drops on the PEGs test compared to the CT genotype.

Rs274622: The results of this SNP were also different by domain. The CC genotype performed better on IDED total error, stage 8 errors, compared to both the CT and TT. Furthermore, they performed better on R-AVLT total words recognized after 30 min delay, and on Similarities subset of WAIS compared to the TT genotype. On the other hand, the CC genotype performed worse on the Digit Span Forward, Reverse subtest of WAIS compared to the CT or TT genotypes. And in the same direction the CC genotype performed worse on the Digit Span total score only compared to the TT genotype. And lastly the CC genotype performed worse on WMI-Age adjusted compared to the CT or TT genotypes.

Rs724226: The results of this SNP were almost identical to the results from rs274622 (The AA genotype corresponding to the CC genotype and the GG genotype corresponding to the TT , in terms of tests and domains, except that this SNP didn't show any significance on the R-AVLT.

This is the first association study that reports an association between these SNPs and cognition, and we don't know if these SNPs have an effect on expression.

APOE

Apolipoprotein E is a polymorphic 299-amino acid protein. The APOE gene resides on chromosome 19 and codes for three alleles: apoE2, apoE3, apoE4. These alleles are a result of two missense single nucleotide polymorphisms (SNPs), rs429358 and rs7412, which result in amino acid changes at residues 112 and 158, respectively. ApoE3 has Cys-112 and Arg158, whereas apoE4 has arginine at both sites, and apoE2 has cysteines (78,79). In our data, having the E2 allele seemed to confer a beneficial effect on cognition. The E2 was associated with better performance on executive functions, psychomotor speed, Language, and intelligence (Matrix reasoning and

Numeracy). Small et al. found statistically significant differences between the E4 and non-E4 groups in global cognitive functioning, episodic memory and executive functioning. In each case the E4 performed more poorly than the non-E4 group (80). Wisdom et al. found that ApoeE4 carriers performed more poorly than apoE4-non carriers on all of the cognitive domains; significant difference were found on global cognitive functioning, episodic memory, executive functioning, and a small effect impacting perceptual speed (81). Our results are supported by the previous literature, and emphasize the importance of APOE in cognition even in normal individuals. We don't have enough E4/E4 individuals to test the pure effect of the E4 allele. Larger studies are warranted.

P-gp

P-glycoprotein (P-gp)/MDR1, a member of the ABC superfamily, is expressed as a result of transcription of the (ABCB1)/MDR1 gene (132). It is known that the ABCB1 gene is highly polymorphic. The three most frequently occurring SNPs are C1236T in exon 12 (dbSNP: rs1128503), G2677T/A in exon 21 (dbSNP: rs2032582) and C3435T in exon 26 (dbSNP: rs1045642) (133). Pg-p relation to cognition in normal people has not been studied previously. However, research has found that P-gp deficiency at the BBB increases β-amyloid deposition in an Alzheimer's disease mouse model (136). And in one study, use of P-gp inducers such as rifampin in mild to moderate Alzheimer's disease patients was associated with less dysfunctional behavior at 3 months. This might be due to increased clearance of β-amyloids due to induction of P-gp (137). It is not surprising that we found some associations between genotype and cognitive domains. The TT genotype of the C1236T performed better on the Rey-Osterrieth Complex Figure Copy, Numeracy, R-AVLT first learning trial of 15 items, and Matrix reasoning. On the other hand, the CC genotype had a higher no. of words recognized after a 30 min delay on R-AVLT and had a higher mean score on the MMSE compared to the TT genotype. The

GA genotype of the G2677A/T performed better on the IGT-total scores, on Rey-Osterrieth Complex Figure test- Copy, R-AVLT (first learning trial of 15 items). However TT genotype performed better on Numeracy compared to GA, GG, or GT genotypes. Carriers of the T allele of C3435T performed better on the WMI[17] compared to the CC. On the other hand the CC genotype performed better on the R-AVLT average no. of words recognized after 30 min delay compared to the CT or TT genotypes. This might indicate that different genotypes lead to differences in expression but it could also mean that P-gp affects different domains differently. Further studies are needed to clarify the role of SNPs in P-gp in cognition, as it is difficult to make any definite conclusions from our data set because of the limited no. of patients and the diverse nature of the cognitive tests.

Our study had a few limitations. We had a relatively small number of patients, which was reduced even further across genotype categories. We didn't correct for the effect of other confounding variables such as supplements, and drugs that our cohort might be using. Although we had a relatively healthy group for their age, they might have been using antihypertensive medications. Also we didn't correct for socioeconomic status, or time of test administration (morning or afternoon).

The SNPs studied need not be responsible for the association seen, as they could segregate with another unstudied polymorphism.

Conclusions

We found that SNPs that affect serotonin, dopamine and glutamate levels in the prefrontal cortex, as well as the efflux pump P-gp, influence cognition in a healthy sample of older adults possibly in a domain specific manner. Thereby identifying a group of people who inherently have lower cognitive functioning in some domains but that is

[17] No age adjustment

still within the normal range. In addition SNPs that were previously associated with lower levels of MPO, thus lower oxidative stress, resulted in higher functioning in the executive functions, verbal memory, verbal IQ and IQ, but not with psychomotor speed. The result of MPO need to be interpreted carefully as the relation of the SNP to enzyme levels is not strongly established, and further complicated by the presence of a binding site for estrogen that enhances expression. APOE E2 allele was associated with higher cognitive performance compared to other alleles which is still in accord to what is known about APOE and Alzheimer's disease.

Our study portrays the complex genetics involved in cognition, but it is also hypothesis generating in terms of the biological roles of putative genes.

The strengths of our study lie in the fact that we studied multiple genes, and we corrected for the effects of those genes. We used a wide battery of neuropsychological tests that covers a wide range of cognitive domains, and we have a fairly homogenous population. However, the age and IQ range of our population is narrow, and our results cannot be generalized outside of these ranges.

Table 3 Primer sequence, PCR, and pyrosequencing conditions for MDR1 (ABCB1), MPO, APOE, COMT and GRM3 SNP analysis.

SNP name	dbSNP accession number	Forward Primer [a] (5'-3')	Reverse Primer [a] (5'-3')	Number of Cycles	Annealing Temperature (°C)	Internal Primer [bc] (5'-3')	Sequence to Analyze [d]
MDR1/ABCC1 C1236T	rs1128503	GTGTCTGTGAATTGCCTTGAAGTT	*GCATGGGTCATCTCACCATCC	45	62	F-TGGTAGATCTTGAAGGG	C/TCTGAACCTGAA
MDR1/ABCC1 G2677T/A	rs2032582	*AGCATAGTAAGCAGTAGGGAGTAACA	CTGGACAAGCACTGAAAGATAAGA	45	62	R-GATAAGAAAGAACTAGAAGG	TG/A/TCTGGGA[e]A
MDR1/ABCC1 C3435T	rs1045642	GAGCCCATCCTGTTTGACTG	*GCATGTATGTTGGCCTCCTT	45	60	F-GGTGGTGTCACAGGA AGA	GATC/TGT
APOE C112R	rs429358	TAAGCTTGGCACGGCTGTCCAAGGA	*ACAGAATTCGCCCCGGCCTGGTACAC	50	68	F-CGCGGACATGGAGGA	CGTGC/TGCGGCCGCCTGG
APOE C158R	rs7412	TAAGCTTGGCACGGCTGTCCAAGGA	*ACAGAATTCGCCCCGGCCTGGTACAC	50	68	F-TGCCGATGACCTGCA	GAAGC/TGCCTGGCAGTG
GRM3 hcv11245618	rs6465084	*TTGCCTTAATGACACAAAGTTCTC	CCGCTGCTCTTTCCATATTGA	50	58	R-TCCATGAAAAAGGCA	CAC/TATTTAAT[e]GGTAATTTG
GRM3 rs917071	rs917071	GCCTGAATTGAAGACTCA	*TCTCCAGCGTATTACTGT	50	47	F-TTGAAGACTCAATTTCCAT	ATTGC/TAGAGTT

Table 3-continued

GRM3 rs274622	rs274622	GGAAACATTGACTGTATCCGA	*ATCCTTTTCTGCTACCACCTC	50	50	F-GCAGGACTAGAGAAGGAC	C/TAATGAGGGGGTG
GRM3 rs724226	rs724226	TCACTTTGTTTCCTGTCA	*GGCATGTTTAGAAAGCAA	50	48	F-TCTTTCTGTAAACACTTGC	TG/ACTCCTCTAC
GRM3 rs1476455	rs1476455	CATTCCCTTCTAGTCTTT	*TGCGTTGAAAGAAAGTGAAAA	50	50	F-AAAATTTTAAATTTTAAAGA	A/CAAATTTAAAAG
GRM3 rs1989796	rs1989796	CATTCCTTGAGTCCTGATTTCTTT	*GTGCAGTGGCTCTCATCAGT	50	56	F-CCTGGATTCAAGCGA	TTC/TTCCTGCCTCA
GRM3 rs1468412	rs1468412	GCACAGTGATATGTTCCTTC	*CCTCCAGTGCAATTTTTATG	50	48	F-GCAATGTTATAGGCAGTA	A/TAATGATTGTTAT
MPO G-129A	rs34097845	AAGAATCGCTTGAACCATTGCA	*ACTGGGGTTGGAAGGTACACACA	45	60	F-TCCCCCCATTTCAGG	A/GGCCCCTCTGTGTGTACCT
MPO C-463T	rs2333227	ATGTTTGCCAGGCTGGTCTT	*TTGGGCTGGTAGTGCTAAATTC	45	60	F-CCTCAAGTGATCCACC	C/TGCCTCAGCCTCCCAAAGTGCTGGGA
COMT Val158Met	rs4680	*GCTCATCACCATCGAGATCA	CTTTTTCCAGGTCTGACAACG	40	55	R-TGCACACCTTGTCCT	TCAC/TGC

a, * = biotin molecule attached; b, R = reverse primer, c, F= forward primer; d, simplex entry nucleotide information for Pyrosequencing; e, assays on reverse complement strand

Table 4 Cognitive domains studied and measures used.

Cognitive Domain	**Measure(s)**
Premorbid Ability	*Wide range achievement test 3(WRAT)*
General Mental Ability	*MMSE*
General Intellectual Ability – Wechsler Adult Intelligence Scale-III	
Full Scale IQ	*Performance IQ* *Block Design* *Matrix Reasoning*
	Verbal IQ *Vocabulary* *Similarities*
Attention/concentration/ Psychomotor Speed	*Digit Symbol*
Psychomotor Speed	*Trail Making Test A(Trail-A)* *Grooved Pegboard Test(PEGs)*
Working Memory	*Working Memory Index(WMI)* Letter Number sequencing (LN) Arithmetic Digit Span (Wechsler Adult Intelligence Scale-III) *Benton Visual Retention Test-Revised(BVRT-R)*
Anterograde Memory (Verbal and Non-Verbal)	*Rey Auditory-Verbal Learning Test(R-AVLT)* *Rey-Osterrieth Complex Figure Test-Delay Condition*
Language	*Controlled Oral Word Association Test(COWA)* *Boston Naming Test (BNT)*
Visuospatial Ability	*Rey-Osterrieth Complex Figure Test-Copy* *Benton Facial Recognition Test*
Executive Functioning	*Trail Making Test B(Trail-B)* *Wisconsin Card Sorting Test (WCST)* *Intra-Dimensional/Extra-Dimensional Shift(ID-ED)* *Iowa Gambling Task (IGT)*
Emotional Functioning	*Beck Depression Inventory (BDI-II)*
Numeracy	*Numeracy*

Table 5 Demographics of older adults

Number of participants	Total N=39 Male (n=19), Female (N=20)
Age (years)	Range (61-91) Mean, SD (77.8, 8.26)
Education (years)	Range (12-20) Mean , SD (16, 2.6)
Full Scale IQ	Range (99-141) Mean , SD (120.4, 9.5)

Table 6 Hardy Weinberg Equilibrium, genotype, and allele frequencies for the healthy older adults study

Polymorphism	Allele frequency (%) N=39			Genotype frequency (%) N=39						P-value
MDR1 C1236T rs1128503	C 36(46.2)	T 42(53.8)		CC 10(25.6)		CT 16(41)		TT 13(33)		0.2755
MDR1 G2677A/T rs2032582	G 37(47.4)	T 38(48.7)	A 3(3.8)	GG 8(20.5)	GT 18(46.2)	GA 3(7.7)	TT 10(25.6)	TA 0(0)	AA 0(0)	0.3346
MDR1 C3435T rs1045642	C 32(41)	T 46 (59)		TT 14(35.9)		TC 18(46.2)		CC 7(17.9)		0.7730
APOE Cys112Arg rs429358	T 72(92.3)	C 6(7.7)		TT 33(84.6)		TC 6(15.4)		CC 0(0)		1
APOE Cys158Arg rs7412	T 7(9)	C 71(91)		TT 0(0)		TC 7(17.9)		CC 32(82.1)		1
GRM3 rs6465084 /hcv11245618	T 64(83.3)	C 14(17.9)		TT 26(66.7)		TC 12(30.8)		CC 1(2.6)		1
GRM3 rs917071	T 15(19.2)	C 63(80.8)		TT 0(0)		TC 15(38.5)		CC 24(61.5)		0.3772
GRM3 rs274622	T 49(62.8)	C 29(37.2)		TT 13(33.3)		TC 23(59)		CC 3(7.7)		0.2183
GRM3 rs724226	A 29(37.2)	G 49(62.8)		AA 3(7.7)		AG 23(59)		GG 13(33.3)		0.2183
GRM3 rs1989796	T 32(41)	C 46(59)		TT 8(20.5)		TC 16(41)		CC 15(38.5)		0.3419
GRM3 rs1468412	T 13(16.7)	A 65(83.3)		TT 1(2.6)		TA 11(28.2)		AA 27(69.2)		1
GRM3 rs1476455	A 9(11.5)	C 69(88.5)		AA 0(0)		AC 9(23.1)		CC 30(76.9)		1
MPO G-129A rs34097845	A 3(3.8)	G 75(96.2)		AA 0(0)		AG 3(7.7)		GG 75(92.3)		1
MPO G463A rs2333227	G 59(75.6)	A 19(24.4)		AA 2(5.1)		GA 15(38.5)		GG 22(56.4)		1
COMT rs4680	C 37(47.4)	T 41(52.6)		TT 8(20.5)		TC 25(64.1)		CC 6(15.4)		0.0747
Serotonin	L 40(50.3)	S 38(48.7)		LL 9(23.1)		LS 22(56.4)		SS 8(20.5)		0.4207

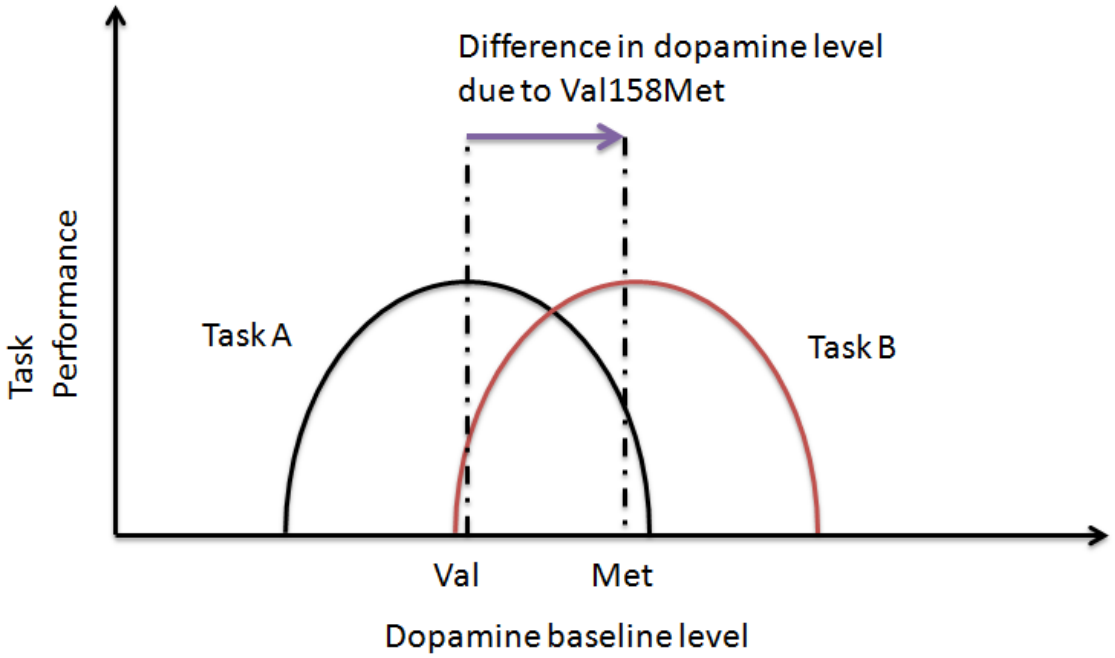

Figure 9 The relationship between dopamine levels and performance on cognitive tasks.

Met= More dopamine, Val= Less Dopamine. Task A in our data: Rey Auditory Verbal Learning test, Digit Span, Similarities, Verbal IQ Task B in our data: Rey Osterrieth Complex figure-Copy, Benton Visual Retention Test, WCST.

Modified from cools et al

CHAPTER 5

GENETIC VARIATION MODULATES COGNITIVE FUNCTION POST CHEMOTHERAPY IN LYMPHOMA PATIENTS

Introduction

Non-Hodgkin lymphoma (NHL) is one of the most common cancers in the United States ranking 7th in cancer incidence. The risk of developing NHL increases throughout life. The average age at diagnosis is in the 60s, and approximately half of patients diagnosed with NHL are older than 65. Survival varies widely by cell type and stage of disease. For NHL, the overall 1- and 5-year relative survival is 81% and 67%, respectively; survival declines to 55% at 10 years after diagnosis (150). With these high survival rates, concerns are rising about the short and long term effects of chemotherapy on brain functions i.e. chemobrain. A growing number of studies are supporting the hypothesis that cancer patients with no central nervous system (CNS) involvement suffer from cognitive deficits after receiving systemic chemotherapy (11)(7)(18)(6)(73). Ahles and his colleagues (7) compared the neuropsychological functioning of long term survivors of breast cancer and lymphoma who had been treated with standard dose chemotherapy or local therapy only (surgery or radiation). They found that survivors who had been treated with systemic chemotherapy scored significantly lower on the battery of neuropsychological tests compared with those treated with local therapy only ($p < 0.04$), particularly in the domains of verbal memory ($P < 0.01$) and psychomotor functioning ($P < 0.03$). Jansen et al. reviewed 16 studies that evaluated cognitive function in chemotherapy patients (18). When compared with normative data, significant effect sizes were found for four domains of cognitive function (executive function, information processing speed, verbal memory and visual memory) (18).

Several candidate mechanisms for chemotherapy-induced cognitive changes have been proposed. These include changes in the blood brain-barrier integrity, DNA damage

and telomere length, cytokine dysregulation, estrogen or testosterone reduction, and genetic susceptibility (25). Polymorphisms in genes that code for transporters, DNA repair enzymes, proteins that maintain neuronal plasticity, neurotransmitters, and antioxidants are likely to influence cognitive change post chemotherapy. The amount of chemotherapeutic agent in the body is also likely to affect cognition. For patients with diffuse large B-cell lymphoma, there is usually a compelling rationale for anthracycline based combination (ABC) chemotherapy including doxorubicin. The factors influencing the disposition of doxorubicin and mechanisms determining efficacy of treatment are influenced by the action of various influx and efflux transporters that are responsible for its translocation across cellular membranes, the drug metabolizing enzymes which are responsible for its biotransformation as well as genes involved in DNA repair which act to counter act its effect. The genes involved in the metabolism of doxorubicin include cytochrome P450 3A4 (CYP3A4), cytochrome P450 3A5 (CYP3A5), the carbonyl reductase 1 (CBR1) gene and the glutathione-S-transferase P1 (GSTP1). Transport related genes include ATP-binding cassette, subfamily B, member 1 (ABCB1/MDR1), ATP-binding cassette, subfamily C, member 1 (ABCC1/MRP1), ATP-binding cassette, subfamily C, member 2 (ABCC2/MRP2), and ATP-binding cassette, subfamily G, member 2 (ABCG2/BCRP). These transporters can transport the drug outside the cell and have been implicated in resistance to anthracycline treatment. DNA repair genes involved in the DNA-adduct repair process include the excision repair cross-complementing rodent repair deficiency, complementation group 2 (ERCC2/XPD); and the mismatch repair genes mutL homolog 1 (MLH1), and mutS homolog 2 (MSH2). The TOP2A gene encodes the enzyme DNA-topoisomerase II alpha (topo II) which is the molecular target of anthracyclines. Topo II is a nuclear enzyme responsible for regulating the level of supercoiling of the double helix which is required for DNA replication and transcription. Doxorubicin acts by inhibiting topo II activity, therefore polymorphisms in the TOP2A gene may alter response to treatment(151). Polymorphisms in genes that are part of the

doxorubicin disposition pathway are likely to have influences on cognition. In addition polymorphisms in drug transporter genes may result in increased penetration of the blood-brain barrier and increased neurocognitive effects.

A majority of the studies that evaluated cognition following chemotherapy were cross-sectional in design, had no pretreatment data, and focused mainly on breast cancer survivors. Little work has been done on other cancer types and even a smaller number were longitudinal in design. In addition, there is a paucity of literature on the effects of various polymorphisms on cognition post chemotherapy, especially in community dwelling NHL patients.

The aims of the present study were 1) to describe the frequency of various SNPs involved in the doxorubicin metabolic pathway and other SNPs proposed to affect cognition, 2) to evaluate the changes in cognitive function and frailty post chemotherapy over time, and 3) to evaluate the contribution of the above SNPs to measures of neurocognitive abilities and frailty among community dwelling NHL patients. We hypothesized that genetic variations modulate the effects of chemotherapy on cognition. Specifically, genetic variations that adversely affect cognitive function in healthy individuals will also predispose to having poorer function post chemotherapy. In addition, genetic variations in genes across the drug disposition pathway will influence drug levels and functionality and affect its delivery to the brain, and affect cognition and frailty among lymphoma survivors.

To our knowledge this is the first longitudinal study of cognitive effects and frailty measures post chemotherapy in NHL patients treated in the community setting.

Methods

This was a cohort study with six-month follow-up for short-term neurocognitive abilities and frailty measures. The study protocol was approved by the University of Iowa Institutional Review Board (IRB ID # 200811742). All participants provided their written

informed consent. Three major approaches to case ascertainment were implemented: (1) an electronic reporting pathology system (ePath); (2) reporting from 10 selected facilities that did not use ePath; and (3) reporting from the University of Iowa Hospitals and Clinics (UIHC).

Patients: Patients were eligible to enroll if they were 45 years or older, Iowa residents at time of diagnosis and initial contact, diagnosed with histologically confirmed diffuse large B- cell lymphoma or grade 3 follicular lymphoma. Patients were excluded if they received prior chemotherapy in the previous 3 years or if they had brain, spinal cord or other CNS involvement.

Study design

Patients completed four cognition and frailty questionnaires. Two questionnaires were administered over the phone and the other 2 were mailed. The patients completed the questionnaires at baseline, before chemotherapy, and after a period of 6 months from baseline, during which they received treatment.

Cognition and frailty measures used

(1) The modified Telephone Interview for Cognitive Status (TICS-M): TICS-M is a brief, reliable, and valid cognitive screening instrument. The TICS-M is a 14-item instrument that assesses global cognition, with an emphasis on learning and memory. The total score ranges from 0 – 50, with higher scores indicating better cognition. Similar to other cognitive screening measures like the Mini Mental State Examination, the TICS-M assesses orientation (e.g., participant's name, telephone number, month date, year, season, day of the week), attention (e.g., counting backwards, serial sevens), and language (e.g., naming, phrase repetition, following simple commands). This measure also emphasizes new learning and memory with immediate recall of a 10-item word list and a delayed recall of that same word list after approximately 5 minutes. Given the total score on this measure is weighted to memory; this instrument might be particularly useful

in identifying cases of early dementia and amnesic mild cognitive impairment associated with cancer and/or cancer treatment (152).

(2) The Multiple Ability Self Report Questionnaire (MASQ): MASQ is a self-report measure comprising items from five cognitive domains: language, visual perceptual ability, verbal memory, visual spatial memory, and attention (153).

(3)The Functional Assessment of Cancer Therapy- General (FACT-G): FACT-G is a 27-item measure divided into four different quality of life domains: Physical Well-Being, Social/Family Well-Being, Emotional Well-Being, and Functional Well-Being. Items are rated on a 0 (not at all) to 4 (very much) point-scale with statements regarding functioning in the past seven days (i.e., I am satisfied with family communication about my illness). It is considered appropriate for use with cancer patients, and has also been validated in chronic illness conditions and in the general population (154).

(4) The Late Life Function and Disability Instrument (LLFDI). LLFDI is a verbally-administered instrument for community-dwelling adults, in which they self-report their capability in physical functioning (155).

Demographic data were gathered from the surveys as well as from medical record data. Medical records were the primary source for information for chemotherapy regimens and complications, tumor size, histology and stage, along with disease status at the end of treatment. Medical record accession and abstraction was conducted by Iowa Cancer Registry staff on-site at the treating facilities.

DNA and genotyping

Patients provided consent for a saliva sample for DNA extraction and subsequent analysis of polymorphisms. SNPs that are involved in the doxorubicin metabolic pathway or associated with cognitive function were determined. The specific SNPs were selected utilizing the HapMap project (http://hapmap.ncbi.nlm.nih.gov/index.html.en), NCBI SNP database (http://www.ncbi.nlm.nih.gov/snp), GeneCards (http://www-

bimas.cit.nih.gov/cards/index.shtml) and Pharmacogenomics Knowledge Base (http://www.pharmgkb.org/). SNPs were selected according to the following criteria: 1- nonsynonymous SNPs predicting alteration of the protein function; 2- SNPs with minor allele frequency in the study population >5% based on frequency (predominantly Caucasian in Iowa) and 3- SNPs in previously published literature. SNPs studied are summarized in Table 7.

DNA was extracted from Saliva (DNA Genotek- OG250 kits) and quantified using real-time PCR-based human DNA quantification kit (Quantifiler®) from Applied Biosystems. Genotypes were determined using Pyrosequencing^{TM}. Polymerase chain reaction (PCR) and pyrosequencing primers were designed using PyrosequencingTM Assay Design version 1.01 software (http://www. pyrosequencing.com). PCR products were visualized by electrophoresis on 2 % agarose gels stained with ethidium bromide prior to genotyping to confirm the correct band size. Sample preparation of the DNA template prior to pyrosequencing was performed using the Vacuum Prep Tool (Biotage, Uppsala, Sweden). Then, Pyrosequencing was carried out using the PyroGold SNP Reagents (Pyrosequencing AB, Uppsala, Sweden), and the PSQ 96MA instrument with accompanying pyrosequencing software (Biotage, Uppsala, Sweden). Repeat sequencing of 40 random samples was done as a quality control. Real time PCR (StepOne Real Time PCR system, Applied Biosystems) was used to obtain the genotype of CYP3A5 and Taqman Genotyper Software V1.0 (Applied Biosystems) was used to assign genotype. Table 8 summarizes PCR and pyrosequencing primers in addition to cycling conditions. It is worthwhile to notice here that a primer mismatch was created for the MPO463 (rs2333227) to increase assay specificity.

Statistical analysis

Statistical analysis was carried out using JMP statistical software version 10.0.0 (SAS Institute Inc., NC, and USA). Descriptive statistics were defined by mean and

standard deviation. The paired t-test was used to compare baseline-follow up data, while the independent t-test with unequal variance was used to compare the data to published normal data. A simple t-test was used to test whether the percent change was different from the hypothesized mean of zero. Several association models were explored which included genotype based model (for example homozygous A (AA), homozygous B (BB) and heterozygous (AB) or allele based model (for example: two copies of A compared to one copy of (A). All possible combinations were explored in pairs. For all pairwise genotype analyses, t-test with equal variance was used when there was no evidence of unequal variance based on the results of Levene's test, while t-test with unequal variance was used otherwise. Genotypes with two categories with less than 3 subjects in any category were excluded from the analysis. To test for Hardy Weinberg equilibrium (HWE) the Chi square or the Haldane Exact Test , provided through the R software, were used when appropriate (R package version 1.4 http://CRAN.R-project.org/package=HardyWeinberg) (140). The false discovery rate method, provided through the q value package from R, was used to adjust for multiple testing, and was set to 0.5 because our study was designed to be exploratory (R package version 1.26.0 http://CRAN.R-project.org/package=qvalue) (141,142). To get the scaled scores of LLFDI, we had to estimate some values on individual patients' scores, which we did using the mean of other scores. In all cognition and frailty tests higher scores indicate better performance.

Results

Sixty five patients were enrolled in the study, and patient characteristics are summarized in Table 9 Fifty six patients provided consent for access and abstraction of medical records, and fifty seven patients provided consent for DNA samples, while 8 patients either refused DNA specimen collection or did not return the specimen container.

Of the fifty six patients that provided consent for medical record abstraction, we have chemotherapy regimen data on fifty four of them. Fifty two received doxorubicin as

part of the regimen. Forty five out of the fifty four received R-CHOP (Rituxiab, cyclophosphamide, doxorubicin, vincristine, prednisone), Three received R-CHOP in addition to etoposide, one received R-CHOP and etoposide and mitoxantrone, one received R-CHOP and mitoxantrone, one received R-CHOP and mitoxantrnoe and cytarabine, and one received rituximab, cyclophosphamide, doxorubicin, vincristine, mitoxantrone and cytarabine. Of the two that didn't receive doxorubicin one received RCE (Rituximab, cyclophosphamide, etoposide) and the other received RCEVP (Rituximab, cyclophosphamide, etoposide, vincristine , and prednisone). SNP frequencies in our population

All SNPs studied were in Hardy Weinberg equilibrium, Table 10 summarizes HWE, genotypes, and allele frequencies.

Changes in cognitive function and frailty post chemotherapy

Table 11 summarizes the means and standard deviations of the cognitive and frailty measures at baseline and at follow-up. Figure 15 represents the percent change from baseline.

1- The modified Telephone Interview for Cognitive Status (TICS-M)

The mean score on the TICS-M at baseline for all participants was 23.45 (N=65; SD 3.52), while the mean score at follow up was 24.13 (N= 55; SD 4.42). There was no statistically significant difference between baseline and follow up (Figure 15). It remained not significant after adjusting for age and sex. Patients who finished high school had an improvement on the scores of TIC-S M (Paired t-test P value 0.0201). The mean score at baseline was 23.24 (N=21; SD 3.37), while the mean score at follow up was 24.88 (N= 17; SD 4.01). When compared to published normative data (156), our cohort performed significantly worse at both baseline and follow up (unequal variance t-

test, P value <0.0001 for both). This significance was maintained after adjusting for sex, and education.

2- The Multiple Ability Self Report Questionnaire (MASQ):

The mean score on the MASQ at baseline for all participants was 156.6 (N=55; SD 24.47), and the mean score at follow up was 155.94 (N=51; SD 29.88). There was no change from baseline on the total MASQ scores (N= 49, Paired t-test, P value 0.4244). There was no change from baseline on the separate MASQ domains. There were no differences between males and females and no effect of age. The category of patients who finished high school had decline on the verbal memory domain (N=15 Paired t-test P value 0.015). The mean score at baseline was 35.5 (N=15; SD 4.29), while the mean score at follow up was 31.9 (N= 17; SD 6.86).

3- The Functional Assessment of Cancer Therapy (FACT-G)

The mean score on FACT-G at baseline for all participants was 77.85 (N=55; SD 16.21), and the mean score at follow up was 84.45 (N=51; SD 16.88). There was a statistically significant improvement from baseline after therapy (N= 49, Paired t-test, P value <0.0001). This change is also clinically meaningful as there is a difference of more than 5 points (157), however, this significance is lost after the age of 70. Looking at each domain separately, we see a significant improvement on 3 domains: Physical Well- Being (N= 49, Paired t-test, P value <0.0001), Emotional Well-Being (N= 48, Paired t-test, P value 0.0485), and Functional Well Being (N= 48, Paired t-test , P value <0.0001). After adjusting for age, the significant improvement on the Physical Well Being domain was lost after the age of 80 years, while the significance on the Functional Well-Being domain was lost after the age of 70 years. There was an improvement on the Emotional

Well Being domain for ages 50-59 years (P value 0.0192), in addition to an improvement on the Social/Family Well Being domain for ages 60-69 years (P value 0.0213).

Females didn't show any significant change from baseline (N=23, P value 0.1841), while males had a significant improvement from baseline (N=26, P value < 0.0001). There was no difference on FACT-G scores between males and females at baseline (unequal variance t-test, P value 0.315), but at follow up, males performed better than females (unequal variance t-test, P value 0.0423). In terms of subscales, both males and females had a significant improvement from baseline on both Physical Well- Being and Functional Well Being domains, but males performed better than females at follow up on the Physical Well-Being domain (P value ; 0.0471). There was no significant improvement from baseline on the Emotional Well-Being and the Social/Family Well-Being domains even after adjusting for gender.

The performance of our cohort on the total score of FACT-G was comparable to published data for normal adults at baseline, but they performed better at follow up (P 0.0318). The performance was similar to published data on cancer patients (157). Compared to the data of normal adults, Physical Well-Being scores were significantly lower at baseline (unequal variance t-test P value 0.0006), but at follow up our patients were better than normal adults (P value 0.0477). Compared to the data published on cancer patients, Physical Well-Being scores were the same at baseline, but at follow up our patients performed better (P value < 0.0001). Social/Family Well- Being scores were better than published data for normal adults at both baseline and follow up (P value < 0.0001), but they were the same as published data for cancer patients. The Emotional Well-Being scores for our patients were the same as published data for normal adults at both baseline and follow up. They were also the same as for the data for cancer patients at baseline, but at follow up, they performed better than cancer patients (P value < 0.0001). And lastly, performance on the Functional-Well Being domain was the same as for the published data on normal adults at baseline, and better than normal adults at follow up (P

value 0.003). Compared to data on cancer patients, they performed worse at baseline (P value 0.0498), but better at follow up (P value 0.0101).

4- The Late Life Function and Disability Instrument (LLFDI)

Adjusted on a scale from 0-100, the mean score for the LLFDI at baseline for all participants was 62.39 (N=65; SD 11.54), while the mean score at follow up was 62.02 (N= 55; SD 13.93). There was no statistically significant difference between baseline and follow up on either the total score or the subscales. It remained not significant after adjusting for age, and education. When the scores were categorized based on sex, females had a reduction in basic lower extremity scores while males didn't (ANOVA P = 0.0071, Female mean -5.35, 95% CI -9.9 to minus 0.786). In general males performed better than females on all the scores of LLFDI at baseline and at follow up except the scores on the basic lower extremity score where they were comparable to females at baseline. Our data are consistent with published normal data on non mobility limited people, the only difference was on the basic lower extremity scale, on which our population reported better functionality at baseline (t-test with unequal variance, P value 0.0166) (155).

Effect of SNPs

1- The modified Telephone Interview for Cognitive Status (TICS-M)

None of the SNPs studied in relation to TICS-M were significant after adjusting for multiple testing.

2- The Multiple Ability Self Report Questionnaire (MASQ):

Although there were no differences from baseline on the MASQ in general, differences were seen when scores were stratified by genotype. People with the AA

genotype of the CYP3A4*1B (n=45, mean difference from baseline -5.7, 95% CI: -13.55 to 2.214) performed worse than the AG genotype (n=3, mean difference from baseline 42.3, 95% CI: 11.81 to 72.85) (equal variance t-test, P value 0.0036, q value 0.3924). This was in the same direction as the percent difference from baseline. People with the AA genotype of the CYP3A4*1B (n=45, mean percent difference from baseline -2.9, 95% CI: -7.74 to 1.842) performed worse than the AG genotype (n=3, mean percent difference from baseline 33.29, 95% CI: 14.75 to 51.836) (equal variance t-test, P value 0.0004, q value 0.0436).

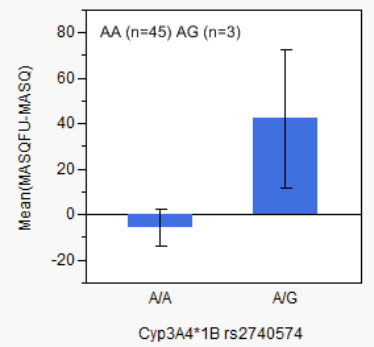

Figure 10 Mean difference on MASQ vs. CYP3A41B genotype

In addition, people with the GG genotype of MPO129 (n=46, mean percent difference from baseline -2.96, 95% CI: -7.93 to 1.99) performed worse than the AG genotype (n=3, mean percent difference from baseline 27.66, 95% CI: 8.22 to 47.1) (equal variance t-test, P value 0.0035, q value 0.1908).

When the subsets of MASQ were studied separately, none of the SNPs were significant after adjusting for multiple testing on the language domain. There was no effect of genotype on difference from baseline on visual spatial memory; however the

genotypes had an effect on the percent difference from baseline. People with the CC genotype of P-gp C1236T (n=15, mean percent difference from baseline -7.2, 95% CI: -12.5 to -1.9) performed worse than the TT genotype (n=11, mean percent difference from baseline 5.2, 95% CI: -1 to 11.39) (equal variance t-test, P value 0.0045, q value 0.4264).

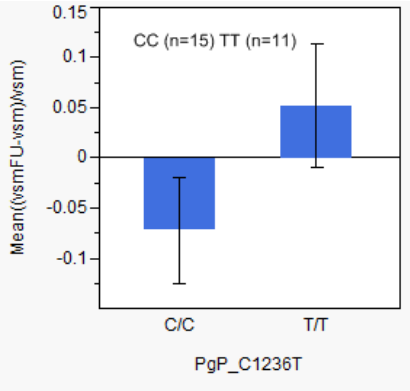

Figure 11 Mean percent change on Visual Spatial Memory domain of MASQ vs. Pgp C1236T genotype (multiply by 100 for percent)

In addition, people with the GG genotype of MPO129 (n=46, mean percent difference from baseline -3.5, 95% CI: -9.4 to 2.34) performed worse than the AG genotype (n=3, mean percent difference from baseline 2.9, 95% CI: 5.86 to 51.65) (equal variance t-test, P value 0.0085, q value 0.4264). One SNP had an effect on the visual perceptual ability. People with the AA genotype of the CYP3A4*1B (n=45, mean difference from baseline -0.69, 95% CI: -2.12 to 0.739) performed worse than the AG genotype (n=3, mean difference from baseline 9.67, 95% CI: 4.14 to 15.2) (equal variance t-test, P value 0.0007, q value 0.0763). On the verbal memory domain one SNP endured adjustments for multiple testing; MPO129 (equal variance t-test, P value 0.0016,

q value 0.1744). This SNP was interesting, for while people with the AG genotype had an increase from baseline (n=3, mean 12.67, 95% CI: 3.87 to 21.46), people with the GG genotype had a decline from baseline (n=46, mean -2.41; 95% CI: -4.7 to - 0.17). No SNPs were significant on the percent change from baseline. On the attention domain, three SNPs had an effect: CYP3A4*1B, CYP3A5*3C rs776746, and TOP2A rs471692. The AG genotype of CYP3A4*1B had a mean improvement from baseline of 10.67 (n=3,95% CI:4.83-16.51), while the AA genotype although not statistically significant had a mean decline of -0.53(n=45, 95% CI: -2.04 to 0.975) (equal variance t-test P value 0.0005 q value 0.047). People with the AA genotype of TOP2A rs471692 had a mean decline of -8.67 (n=3, 95% CI: -14.7 to -2.63), and people with the AG/GG genotypes had a mean change of 0.72 (n=46, 95% CI: -.82 to 2.26) this change was not statistically different from zero (equal variance t-test P value 0.0039 q value 0.1221). Those two SNPs had similar direction effect on the percent change from baseline.

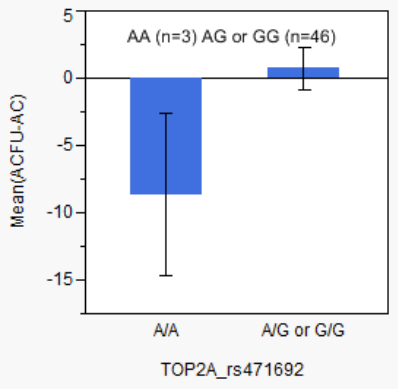

Figure 12 Mean difference on Attention domain of MASQ vs. TOP2A rs471692 genotype

CYP3A5*3C didn't affect the mean difference from baseline but affected the percent difference. People with the AA or AG of the CYP3A5*3C genotype had a mean percent improvement of 18% (n=6, 95% CI: 2.85 to 33%) while people with the GG genotype had a mean percent change of -0.2% (n=43, 95% CI: -5.9% to 5.4%, equal variance t-test P value 0.0281 q value 0.4386) which was not significantly different from zero.

3- The Functional Assessment of Cancer Therapy
(FACT-G)

No differences were found across genotypes on the total score of FACT-G. However, three SNPs had an effect on the scores of the Physical Well-Being domain. The first one was TOP2A rs471692. The GG genotype (n=35) had an improvement over baseline while the AG/AA didn't n= 14 (equal variance t-test P value 0.023, q value 0.3857). The mean difference from baseline for the GG genotype was 4.85, (95% CI: 3.4 to 6.26), while the AG/AA mean difference was 1.76 (95% CI: -0.47 to 3.99). The second SNP was the P-gp C3435T rs1045642. People with the TT genotype of Pg-p C3435T (n=15) had a higher improvement from baseline compared to the CC/CT genotype (n=34, unequal variance t-test P value 0.0174, q value 0.3502). The mean difference for the TT genotype was 6.5 (95% CI: 3.77 to 9.23), while the mean difference for CC/CT was 2.85 (95% CI: 1.59 to 4.1). The third SNP was MLH1 rs1800734. People with the GG genotype (n=35) had a significant change from baseline while people with the AG/AA genotype (n=14) didn't have any significant change (equal variance t-test P value 0.0039, q value 0.1811). The mean difference for the GG genotype was 5.1(95% CI: 3.7 to 6.4) while the mean difference for the AG/AA genotypes was 1.21 (95% CI:-0.94 to 3.37). None of those SNPS had an effect on the percent change from baseline after adjusting for multiple testing.

None of the SNPs had an effect on the change or percent change form baseline on the Social/Family Well-Being domain.

One SNP had an effect on the Emotional Well-Being domain. The A/C genotype (n=9) of the GRM3 rs1476455 was associated with improvement in Emotional Well-Being after therapy compared to the CC genotype (n=39) (equal variance t-test P value 0.0025, q value 0.2575). The mean difference for the AC genotype from baseline was 3.3 (95% CI: 1.59 to 5.08), while the CC genotype mean change was 0.26 (95% CI: -0.58 to 1.1). GRM3 rs147455 was even more significant on the percent change from baseline (equal variance t-test P value 0.001, q value 0.104) with the AC genotype having a mean percent change of 22.9% (95% CI: 12.3% to 33.6%) compared to the CC genotype percent change of 2.4% (95%CI: -2.8% to 7.45%).

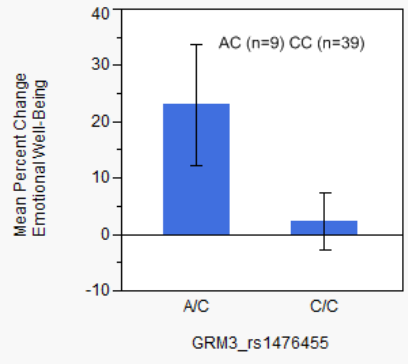

Figure 13 Mean percent change on Emotional Well-Being domain of FACT-G vs. GRM3 rs1476455 genotype

Three SNPs had an effect on the scores of the Functional Well-Being domain. The first one was MLH1 rs1800734 (unequal variance t-test P value 0.0061, q value 0.4326). The mean difference from baseline for the GG genotype (n=34) was 4.6 (95% CI: 3.01 to

6.2), while there was no statistically significant difference for the AA/AG genotypes (n=14, mean difference 1.1, 95% CI: -0.87 to 3.06). This SNP had the same effect on the percent change from baseline.

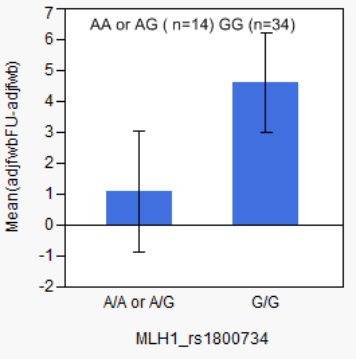

Figure 14 Mean difference on Functional Well-Being domain of FACT-G vs. MLH1 rs1800734 genotype

The second one was ERCC2 156 (equal variance t-test P value 0.018, q value 0.3708). People with the AA genotype (n=13, mean percent difference 28.1, 95%CI: 16.89 to 39.4) had a higher percent improvement from baseline compared to the AC/CC genotypes (n=35, mean percent difference 12.07, 95% CI: 5.2 to 18.9). The last SNP is carbonyl reductase rs100569. People with the TT genotype (n=11, mean percent difference 26.7, 95%CI: 15.8 to 37.6) had a higher percent improvement from baseline than the GT group (n=20, mean percent difference 9.4, 95% CI: 1.3 to 17.5) (equal variance t-test P value 0.0341, q value 0.3708).

4- The Late Life Function and Disability Instrument (LLFDI)

When the difference from baseline and the percent difference of the LLFDI scores were compared across genotypes, none of the SNPs were significant after adjusting for multiple testing. There were differences on the subscales however. Two SNPs had an influence on basic lower extremity score. People with the GG genotype of carbonyl reductase 1 (n=19, mean percent difference from baseline -8.4, 95% CI: -15.7 to -1.1) showed a decline in function compared to the GT group which showed an improvement in function (n=21, mean percent change from baseline 6.9, 95% CI: 0.05 to 1.3) (equal variance t-test P value 0.0037, q value 0.2702). And although there were no significant differences from baseline, genotypes of Glutathione-S-transferase were different from each other (equal variance t-test P value 0.024, q value 0.497). People with the CC genotype (n=9, mean percent difference from baseline -9.2, 95% CI: -18.7 to 0.33) showed a decline although not significant compared to the CT genotype which showed an improvement (n=18, mean percent difference from baseline 4.8, 95% CI: -1.9 to 1.2). None of the SNPs were significant after adjusting for multiple testing when the difference and the percent difference from baseline were studied across genotypes for, the upper extremity score, and advanced lower extremity score.

Discussion

Even though we didn't find any effect of chemotherapy on cognition as measured on the TICS-M, our patients performed significantly worse than published normal data. This is not a contradictory issue, as emerging evidence suggests cancer itself may predispose patients to cognitive impairment (158). The results from MASQ were consistent with what was previously published. Changes were seen in the domains of verbal memory, visual perceptual memory, and attention, but in contrast to the published literature we saw those changes only when we compared scores across genotypes. Results

from the FACT-G were consistent with improvement in functionality after treatment. Social/Family Well-Being scores were the same as published data for cancer patients. This was expected as cancer patients usually have better social/family support (157).

Both CYP3A4 and CYP3A5 are involved in the metabolism of doxorubicin (159) and thus may influence how doxorubicin impacts the brain. Although the SNP rs2740574 of the CYP3A4 has been well studied, studies relating this SNP to enzyme expression levels were inconsistent (160). On the other hand, people with a CYP3A5*1(A) allele of CYP3A5 rs776746 produce high levels of the full-length mRNA and express CYP3A5. Those with the CYP3A5*3(G) allele have sequence variability that creates a splice site with a premature stop codon and don't express CYP3A5 (161). In our dataset patients with the AG genotype of the CYP3A4 had improvement on the total score of MASQ as well as the visual perceptual and attention domains of the MASQ while the AA genotype didn't have any change. Patients with CYP3A5 expresser genotypes (CYP3A5*1/*1; AA and *1/*3; AG) had statistically significant improvement on attention from baseline compared to the non-expresser genotype 3*/3*(GG) which didn't have any change. This could mean that the former have lower levels of the drug, and hence there is less effect on the brain, but it could also mean that those people have better elimination of toxic substances that affect the brain in general.

An important determinant of cognition is neurotransmission. Type-three metabotropic glutamate receptor (GRM3) has been studied in schizophrenia and polymorphisms have been associated with cognitive functioning. It is expressed in astroglial cells, where it regulates expression of the glial glutamate transporter EAAT2. EAAT2-mediated glutamate uptake is critical in regulating glutamate neurotransmission. Polymorphisms in GRM3 have been associated with changes in verbal list learning and verbal fluency (116,117). Two polymorphisms (rs1989796 and rs1476455) were recently related to the presence of refractory global symptoms of schizophrenia. Participants in that study who had an rs1476455 CC genotype had significantly higher refractory

symptoms as measured by the brief psychiatric rating scale than A-carriers (55.1+-10.4 vs. 48.3+-9.2; P value=0.0071)(120). In our study, rs1476455 was related to Emotional Well Being, with the AC genotype performing better then the CC genotype, which is in line with the previous study.

P-glycoprotein (P-gp), a 170 kDa membrane bound efflux pump at the apical membrane of endothelial cells, functions as part of the blood brain barrier (BBB). The Multi Drug Resistance gene (ABCB1) encodes for P-gp, and the three most frequently occurring SNPs in the ABCB1 gene are C1236T in exon 12, G2677T/A in exon 21 and C3435T in exon 26 (136). It is likely that polymorphisms in the ABCB1 gene will affect cognition by changing the clearance of toxic substances. For example ABCB1 has been linked to amyloid clearance in Alzheimer's disease (162). The C3435T in the MDR1/ABCB1 has been studied extensively. The presence of the homozygous T allele has been shown to correlate with reduced MDR1 expression (163). In our study the TT genotype of the C3435T performed better on the Physical Well-Being domain, and the CC genotype of the C1236T performed worse on the visual spatial memory domain. It is contradicting that the T allele which has previously been associated with lower expression, although not consistently, would be associated with better physical functioning and visual spatial memory. A possible explanation would be that people would have compensatory mechanisms such as a higher functioning CYP3A4 and CYP3A5. However this was not the case in our cohort. People with the TT genotype of C3435T had also the non expresser GG genotype of CYP3A5 and had the AA genotype of CYP3A4 (15 people) both of which also performed worse in our study.

There has been increasing evidence that relates oxidative damage, DNA repair and cognition. In their paper, Keller et al. showed that patients who had been diagnosed with mild cognitive impairment had elevated levels of oxidized compounds in the brain at autopsy, and that the levels of oxidized proteins increased as delayed verbal memory performance declined(31). Myeloperoxidase is an enzyme which produces oxidative free

radicals and has been detected in microglia. Hoy et al. described the polymorphisms G-129A (rs34097845) which was significantly associated with serum MPO concentrations, the A allele being associated with lower levels (121). Interestingly enough, MPO129 GG allele in our data, which should correspond to higher levels of MPO and thus higher oxidative damage, showed declines in verbal memory domain of MASQ after chemotherapy compared to the AG genotype that had improvement. In addition, the AG genotype performed better on the visual spatial memory and the total percent change of MASQ.

DNA damage is another possible mechanism for cognitive decline as described by Ahles et al. (25). Chemotherapy might cause cognitive changes through DNA damage caused directly by the cytotoxic agents or through increases in oxidative stress. Topoisomerase II a (TopIIa) is a nuclear enzyme responsible for regulating the level of supercoiling of the double helix which is required for DNA replication and transcription. Doxorubicin acts by inhibiting topo II activity, therefore polymorphisms in the TOP2A gene may alter response to treatment (151). The SNP rs471692 is a boundary non coding SNP, 11 bases downstream from the second exon of the gene *TOP2A* (http://www.ncbi.nlm.nih.gov/snp/). To our knowledge, this SNP was not studied before, and thus we don't have information on the effects of this SNP on enzyme function and doxorubicin binding. This SNP was associated with attention as well as the Physical Well Being component of the FACT-G, the AA genotype being associated with lower performance on attention and the GG genotype being associated with better performance on the Physical Well-Being domain. Another important DNA repair gene is the MLH1. MLH1 is a part of the DNA mismatch repair system MMR. MMR removes nucleotides mis-paired by DNA polymerases and insertion/deletion loops that result from slippage during replication of repetitive sequences or during recombination (28). A frequent a single-nucleotide polymorphism is encountered in the promoter of *MLH1* at position -93 nucleotides from the adenine residue of the start codon 293G>A (rs1800734) (164). This

SNP was associated with gene methylation and epigenetic silencing, the A allele being methylated (165). The association of the GG allele in our study with better Physical-Well Being and Functional Well-Being suggests a role for DNA repair in the physical functioning, but this is not surprising as there is evidence of the influence of genetic factors on physical functioning (166). Excision repair cross-complementing group 2 (*ERCC2*)/Xeroderma pigmentosum complementation group D (*XPD*), is DNA repair enzyme, involved in nucleotide excision repair and basal transcription. The variant A-allele of XPD 156 was associated with increased risk of adenocarcinoma of the lung (AA/AC versus CC; adjusted OR=1.65; 95% CI=1.09–2.50) (*P*=0.02). Furthermore, the presence of one or two variant A-alleles was associated with increased risk for lung cancer (OR=2.49; 95% CI=1.10–5.64) (*P*=0.03) and adenocarcinoma of the lung (OR=5.60; 95% CI=1.52–20.56) (*P*=0.005) among never-smokers only in a north eastern Chinese population (167). In our cohort, the AA allele had a better score on Functional Well Being compared to the AC genotype of XPD 156.

Carbonyl reductases belong to a class of oxidoreductase proteins they catalyze the NADPH reduction of a large number of biologically and pharmacologically active substrates including doxorubicin, and thus polymorphisms in the gene might alter the level and functionality of the protein, and consequently influence the levels of doxorubicin and/or its metabolites. CBR1 is the major carbonyl reductase and is expressed widely in different tissues. The influence of the SNP rs1005696 on enzyme function and expression is not known as this SNP was not studied before. However in our study we found that this SNP affects lower extremity functionality as measured by the LLFDI post chemotherapy (168).

It is important to emphasize here that the polymorphisms studied need not be directly responsible for the association seen, as they could co-segregate with another close by, unstudied polymorphism.

There were a few limitations in our study. Although we had a longitudinal design, we didn't have a control group to compare the results of self reported questionnaires to. We attempted to evaluate cognitive performance of older adults living in the community, and we used self-assessment of cognition, this itself is a limitation, as the connection between self evaluation and objective assessment of cognition is not well understood (169). We found moderate correlations between measures of function and measures of cognition (data not shown).We were also restricted by the sample size. A larger sample size that allows for a minimum of 10 subjects per category is needed. Other factors affect cognition in addition to the variables that we studied, including but not limited to anemia, depression, and the use of antipsychotic drugs and other drugs, see Table 12 for a list of these drugs. The effect that we saw might also be due to a combination of the effect of the chemotherapy regimen that the patients were taking.

Those factors need to be evaluated and controlled for. In addition, our population was predominantly Caucasian, thus the results of this study cannot be generalized. Because we were looking at medical records, we were restricted by the type of clinical data that we obtained. On the other hand, our study is important because it explores the influence of multiple SNPs on cognition post chemotherapy, something that has received little attention.

Conclusions

We determined relevant polymorphisms related to cognitive function and frailty from a community dwelling population. Contrary to our hypothesis, patients showed improvements in physical function after chemotherapy and no change in cognitive function. However, using patient stratification based on genotype, specific groups of patients had a measurable decline in cognitive function post chemotherapy. Cognitive declines were detected in the visual spatial memory domain, and attention domain of the MASQ. Interestingly a SNP in the DNA replication enzyme and the target of doxorubicin

topoisomerase II was associated with varying degree of self reported attention; specifically the AA genotype of rs471692 was associated with statistically significant decline in attention post chemotherapy. This indicates that cognitive changes following chemotherapy can be subtle, and stratification by genotype helps us in identifying susceptible individuals and provides some insights on the inconsistencies that are frequently reported in the literature. We also identified genotypes related to cognitive improvement post chemotherapy. For example the TT genotype of Pgp C3435T was associated with a higher rate of improvement in function compared to the CC or CT genotype.

Cleary, a formal assessment of cognition using objective measures is needed to confirm our results and to further understand the contribution of genetic polymorphisms to cognition. Our research is mainly exploratory. If our results were replicated in a larger study, this will allow us to select patients who are susceptible to cognitive declines post chemotherapy, and would allow us to target cognitive therapies to those patients in hopes of improving their quality of life.

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Table 7 SNPs studied for the cognition post chemotherapy study

Gene	dbSNP accession number	Polymorphism	Amino acid change or position
ABCB1/MDR1	rs1128503	C>T C1236T	G [Gly] ⇒ G [Gly]
ABCB1/MDR1	rs1045642	C>T C3435T	I [Ile] ⇒ I [Ile]
*ABCB1/MDR1	rs2032582	T>A T>G (G2677A/T)	S [Ser] ⇒ T [Thr] S [Ser] ⇒ A [Ala]
Apolipoprotein E (APOE)	rs429358	T>C	C [Cys] ⇒ R [Arg] position 112
Apolipoprotein E (APOE)	rs7412	T>C	R [Arg] ⇒ C [Cys] Position 158
*Glutamate receptor, metabotropic 3	rs6465084 (hcV11245618)	C>T	Intronic
Glutamate receptor, metabotropic 3	rs917071	C>T	Intronic
Glutamate receptor, metabotropic 3	rs274622	C>T	Intronic
Glutamate receptor, metabotropic 3	rs724226	A>G	Intronic
Glutamate receptor, metabotropic 3	rs1989796	C>T	Intronic
Glutamate receptor, metabotropic 3	rs1468412	A>T	Intronic
Glutamate receptor, metabotropic 3	rs1476455	A>C	Intronic
*Glutathione-s-transferase GSTP1	rs1695	T>C	I [Ile] ⇒ V [Val] Position 105
Excision repair cross-complementing 2 ERCC2 (XPD)	rs13181	T>G	K [Lys] ⇒ Q [Gln] Position 751
Excision repair cross-complementing 2 ERCC2 (XPD)	rs238406	A>C	R [Arg] ⇒ R [Arg] Position 156
Topoisomerase 2 alpha (TOP2A)	rs471692	A>G	Intronic
Topoisomerase 2 alpha (TOP2A)	rs13695	A>G	UTR-3 3 prime untranslated region

Table 7-continued

CYP3A4*1B	rs2740574	A>G	Near gene 5
CYP3A5*3C	rs776746	A>G	Intronic
Carbonyl reductase CBR1	rs9024	A>G	UTR-3
*Carbonyl reductase CBR1	rs1005696	G>T	Intronic
ABCC1/MRP1	rs35597	A>G	Intronic
ABCC1/MRP1	rs66657812/rs45511401	G>T	G [Gly] ⇒ V [Val]
ABCC2/MRP2	rs17222723	T>A	V [Val] ⇒ E [Glu]
*ABCC2/MRP2	rs8187710	T>C	C [Cys] ⇒ Y [Tyr]
*ABCG2/BCRP	rs2231142	G>T	Q [Gln] ⇒ K [Lys]
MutL homolog 1(MLH1)	rs1799977	A>G	I [Ile] ⇒ V [Val]
MutL homolog 1(MLH1)	rs1800734	A>G	Near gene 5
*MutS homolog 2(MSH2)	rs3771281	A>G	Intronic
Myeloperoxidase (MPO129)	rs34097845	A>G	Near gene 5 Promoter region
Myeloperoxidase (MPO463) Also known as -642 G>A or -643	rs2333227	C>T	Near gene 5

*SNP on reverse strand

Table 8 Primer sequence, PCR, and pyrosequencing conditions for the cognition post chemotherapy study

SNP	Forward Primer [a](5'-3')	Reverse Primer [a](5'-3')	Number of Cycles	Annealing temperature (°C)	Internal Primer [bc] (5'-3')	Sequence to analyze[d]
GSTP1 rs1695 (I105V)	*GGTGAATGACGGCGTGGA	CCCTTTCTTTGTTCAGCCCC	45	60	R-TTGGTGTAGATGAGGGA	GAC/TGTATTTG[e]
ERCC2 K751Q	GTCACCAGGAACCGTTTATG	*CCTGGAGCAGCTAGAATCAGA	45	58	F-GCAATCTGCTCTATCCTC	T/GCAGCGT
ERCC2 R156R	*CTGCCCTCCAGTAACCTCAT	TGAAGAGTGGTTGGGTTTCC	40	56	R-CCTGCCCCACTGCCG	A/CTTCTATGAGGTTACTGGAGGGCAGG[e]
Top2A rs471692	CAGAACATGGACCCAGGTAAAT	*TTCTCGGTGCCATTCAACAT	45	55	F-TGGACCCAGGTAAATAAT	TA/GTGGATTTCTTTTTTAGGTTTGTGAT
Top2A rs13695	GCTCATGTTCTTCATCTTCTCA	*AAATGTTGTCCCCGAGTCTTC	45	55	F-CTCAAATCATCAGAGGC	CA/GAAGAAAAACACTTTGGCTGTGTCTA
CYP3A4*1B rs2740574	GCACACTCCAGGCATAGGTAA	*GTGGAGCCATTGGCATAAAA	45	57	F-CCATAGAGACAAGGGCA	A/GGAGAGAGGCGATTTAATAGATTTTA
CBR1 rs9024	CCCCATTTTGTACCTTGTCCT	*CCTGCATCAGAGGAAATCACA	45	55	F-CTTATCAATTAGCACTCACT	AATA/GTACTACTAATTGAGCAACCTACGCA

Table 8-continued

CBR1 rs1005696	*CCTGAGGCAAAATGGCACATAT	TTCTCTTTGGGGCTTGATTTG	45	56	R-ACTGACCTCTGTGCTTT	G/TTCTCCTGCCAGCTGATATGTGCCAT[e]
ABCC1 rs35597	GGTAGGCAGGAAGAGCAGGTAA	*CCTGTCCCCTAGGTGCCTTTT	45	60	F-ATTCATTGGTTTTCCAC	A/GTTTCTCAGAAAAGGCACCTAGGGGA
ABCC1 rs66657812/rs45511401	GTTGTGTCGTTTCAGCATCACC	*ACGTGCCCCTCCACTTTGTC	45	60	F-TCTCCATCCCCGAAG	G/TTGCTTTGGTGGCCGTGGTGGCCAG
ABCC2 rs17222723	GCAGCGATTTCTGAAACACAA	*CTCCCACCGCTAATATCAAACA	45	57	F-GATTTCTGAAACACAATGA	GGA/TGAGGATTGACACCAACCAGAAATGT
ABCC2 rs8187710	*TTCCTTGTTTCAGGGTAATGGT	GCCTTCTGCTAGAATTTTGTGC	45	55	R-TCTTCAGGGCTGCCG	C/TACTCTATAATCTTCCC[e]
ABCG2 rs2231142	*ATGATGTTGTGATGGGCACTC	TTGAATGACCCTGTTAATCCG	45	56	R-GAAGAGCTGCTGAGAACT	G/TTAAGTTTTCTCTCACCGTCAGAGTG[e]
MLH1 rs1799977	GTTTATGGGGGATGGTTTTGT	*CATACCGACTAACAGCATTTCC	45	54	F-GGACAATATTCGCTCC	A/C/GTCTTTGGAAATGCTGTTAGTCGGTA

Table 8-continued

MLH1 rs1800734	GGCTGGATGGCGTAAGCTACA	*CGCCAGAAGAGCCAAGGAAA	45	60	F-GATGGCGTAAGCTACA	GCTA/GAAGGAAGAACGTGAGCACGAGGCAC
MSH2 rs3771281	*CATGGTCCTTTGTTTTGAAACT	AACGAAATCGGACTGATCTGA	45	54	R-CCACAATGAAAGACTGC	A/GAACAATCAGTGATCAAAGTTTCAAA[e]
Cyp3A5*3	Real Time PCR: Applied Biosystems Taqman® Allelic Discrimination Assay no. C_26201809_30-					

a, * = biotin molecule attached; b, R = reverse primer, c, F= forward primer; d, simplex entry nucleotide information for Pyrosequencing; e, assays on reverse complement strand

Forward primer mismatch was created

Table 9 Demographics and patient characteristics

Gender	**N total = 65**
Females	33(50.8%)
Males	32(49.2%)
Age in years	
45-49	2 (3.1%)
50-59	16 (24.6%)
60-69	21 (32.3%)
70-79	18 (27.7%)
80+	8 (12.3%)
Education	
Grade school	4(6.2%)
Some high school	4(6.2%)
GED	1(1.5%)
High school	21(32.3%)
Some vocational, business, or trade school	3(4.6%)
At least one full year of college	19(29.2%)
Four year college degree or more	13(20%)
Race	
White	63(97%)
Black/African American	1(1.5%)
American Indian/Alaska Native	1(1.5%)
Histology	N=54
9680/3 Malignant lymphoma, large B-cell, diffuse, centroblastic, NOS	49(90.7%)
9698/3 Follicular lymphoma, grade 3	3(5.6%)
Other*	2(3.7%)
Stage	**N= 52**
Stage I	9(17.3%)
Stage II	8(15.4%)
Stage III	12(23.1%)
Stage 4	23(44.2%)

Table 9-continued

Disease Status	**N= 51**
Complete response	40(78.4%)
Partial response / stable disease	5(9.8%)
Not Evaluated	3(5.9%)
Unknown	3(5.9%)
B symptoms	**N=56**
Yes	15(26.8)
No	41(73.2%)
Tumor size measured in cm^2	**N=32**
Mean	6.23
SD	3.63
Minimum	1.5
Maximum	16

*One patient had both diffuse large B-cell lymphoma & Burkett's Lymphoma and the other had both diffuse large B-cell lymphoma and Follicular Lymphoma)

Table 10 Hardy Weinberg Equilibrium, genotype, and allele frequencies for the cognitive post chemotherapy study

Polymorphism	N	Allele frequency N (%)			Genotype frequency (%)						p-value
MDR1 C1236T rs1128503 ABCB1	57	C	T		CC		CT		TT		0.5426
		62	52		18		26		13		
		54.4	45.6		31.6		45.6		22.8		
MDR1 C3435T rs1045642	57	C	T		TT		TC		CC		0.4081
		49	65		17		31		9		
		43.0	57.0		29.8		54.4		15.8		
MDR1 G2677A/T rs2032582	57	G	T	A	GG	GT	GA	TT	TA	AA	0.9523
		64	48	2	19	25	1	11	1	0	
		56.1	42.1	1.8	33.3	43.9	1.8	19.3	1.8	0.0	
APOE Cys112Arg rs429358	57	T	C		TT		TC		CC		1.0000
		95	19		39		17		1		
		83.3	16.7		68.4		29.8		1.8		
APOE Cys158Arg rs7412	57	T	C		TT		TC		CC		1.0000
		7	107		0		7		50		
		6.1	93.9		0.0		12.3		87.7		
GRM3 rs6465084 /hcv11245618 Reverse strand	57	T	C		TT		TC		CC		0.6771
		92	22		36		20		1		
		80.7	19.3		63.2		35.1		1.8		
GRM3 rs917071	57	T	C		TT		TC		CC		0.7464
		27	87		4		19		34		
		23.7	76.3		7.0		33.3		59.6		
GRM3 rs274622	57	T	C		TT		TC		CC		0.5475
		77	37		27		23		7		
		67.5	32.5		47.4		40.4		12.3		
GRM3 rs724226	57	A	G		AA		AG		GG		0.5678
		40	74		8		24		25		
		35.1	64.9		14.0		42.1		43.9		

Table 10-continued

GRM3 rs1989796	57	T	C	TT	TC	CC	0.8646
		53	61	12	29	16	
		46.5	53.5	21.1	50.9	28.1	
GRM3 rs1468412	57	T	A	TT	TA	AA	1.0000
		27	87	3	21	33	
		23.7	76.3	5.3	36.8	57.9	
GRM3 rs1476455	57	A	C	AA	AC	CC	1.0000
		11	103	0	11	46	
		9.6	90.4	0.0	19.3	80.7	
GSTP1 rs1695 (I105V) Reverse strand	57	T	C	TT	CT	CC	0.1308
		73	41	26	21	10	
		64.0	36.0	45.6	36.8	17.5	
ERCC2 rs13181 (K751Q)	57	T	G	TT	TG	GG	0.7837
		70	44	21	28	8	
		61.4	38.6	36.8	49.1	14.0	
ERCC2 rs238406 (R156R)	57	C	A	AA	AC	CC	0.1451
		57	57	17	23	17	
		50.0	50.0	29.8	40.4	29.8	
Top2A rs471692	57	A	G	AA	AG	GG	0.2156
		22	92	4	14	39	
		19.3	80.7	7.0	24.6	68.4	
Top2A rs13695	57	A	G	AA	AG	GG	0.5994
		31	83	5	21	31	
		27.2	72.8	8.8	36.8	54.4	
CYP3A5 *3C rs776746	57	A	G	AA	AG	GG	0.0566
		9	105	2	5	50	
		7.9	92.1	3.5	8.8	87.7	
CYP3A4*1B rs2740574	57	A	G	AA	AG	GG	0.1746
		109	5	53	3	1	
		95.6	4.4	93.0	5.3	1.8	

Table 10-continued

CBR1 rs9024	57	A	G	AA	AG	GG	1.0000
		14	100	1	12	44	
		12.3	87.7	1.8	21.1	77.2	
CBR1 rs1005696	57	T	G	TT	TG	GG	0.4271
		49	65	12	25	20	
		43.0	57.0	21.1	43.9	35.1	
ABCC1 rs35597	57	A	G	AA	AG	GG	0.6976
		55	59	14	27	16	
		48.2	51.8	24.6	47.4	28.1	
ABCC1 rs66657812/rs45511401	57	T	G	TT	TG	GG	1.0000
		10	104	0	10	47	
		8.8	91.2	0.0	17.5	82.5	
ABCC2 rs17222723	57	T	A	TT	AT	AA	1.0000
		108	6	51	6	0	
		94.7	5.3	89.5	10.5	0.0	
ABCC2 rs8187710 Reverse strand	57	T	C	TT	CT	CC	1.0000
		6	108	0	6	51	
		5.3	94.7	0.0	10.5	89.5	
ABCG2 rs2231142	57	T	G	TT	GT	GG	0.8065
		14	100	0	14	43	
		12.3	87.7	0.0	24.6	75.4	
MLH1 rs1799977	57	A	G	AA	AG	GG	0.3991
		70	44	23	24	10	
		61.4	38.6	40.4	42.1	17.5	
MLH1 rs1800734	57	A	G	AA	AG	GG	0.6909
		17	97	2	13	42	
		14.9	85.1	3.5	22.8	73.7	
MSH2 rs3771281	57	A	G	AA	AG	GG	0.9919
		40	74	7	26	24	
		35.1	64.9	12.3	45.6	42.1	

Table 10-continued

MPO G-129A rs34097845	57	A	G	AA	AG	GG	1.0000
		4	110	0	4	53	
		3.5	96.5	0.0	7.0	93.0	
MPO 463 rs2333227	57	C	T	TT	CT	CC	0.2929
		91	23	4	15	38	
		79.8	20.2	7.0	26.3	66.7	

Table 11 Means and standard deviations of cognitive and frailty measures at baseline and at follow-up, and mean difference between baseline and follow-up.

Measure	**Subscales**	**Baseline Mean(SD), N**	**Follow-up Mean(SD), N**	**Difference Mean(SD), N**	**P value #**
TICs-M	No subscales	23.4(3.52), 65	24.13(4.42), 55	0.509(3.23), 55	0.2477
MASQ		156.6(24.47), 55	155.94(29.88), 51	-3.29(28.54), 49	0.4244
	Language	34.41(5.89), 55	33.65(6.83), 51	-1.14(7.18), 49	0.2709
	Visual-Perceptual	25.7(4.29), 55	26(5.26), 51	-0.122(5.32), 49	0.8727
	Verbal Memory	31.98(6.7), 55	31.2(7.76), 51	-1.48(8.3), 49	0.2170
	Visual Spatial Memory	32.7(5.34), 55	32.41(7.69), 51	-0.67(6.57), 49	0.4762
	Attention	31.78(5.77), 55	32.68(5.77), 51	0.14(5.6), 49	0.8595
FACT-G		77.85(16.21), 55	85.45(16.88), 51	7.16(11.8), 49	<0.0001*
	Physical Well Being	20.1(5.19), 55	23.87(3.96), 51	3.97(4.34), 49	<0.0001*
	Social/Family Well Being	23.04(5.23), 55	23.11(6.44), 51	-0.37(5.54), 49	0.9047
	Emotional Well Being	19.56(3.85), 55	20.71(2.92), 50	0.83(2.85), 48	0.0485*
	Functional Well Being	17.24(6.05), 55	21.08(5.68), 50	3.59(4.52), 48	<0.0001*
LLFDI		62.39(11.54), 65	62.02(13.93), 55	-0.59(8.98), 55	0.6287
	Upper Extremity Score	79(12.75), 65	77.48(16.44), 55	-1.99(12.24), 55	0.2342
	Basic Lower Extremity Score	78.2(17.08), 65	77.39(19.23), 55	-0.97(12.99), 55	0.5838
	Advanced Lower Extremity score	50.98(19.61), 65	52.34(21.93), 55	1.07(12.75), 55	0.5352

P value represents the result of paired t-test between baseline and follow-up.

Table 12 A list of other drugs patients were taking at baseline or follow-up or both.

acetaminophen	carvedilol	fluconazole	lovastatin	pravastatin
acetaminophen-codeine	celecoxib	fluoride topical	medroxyprogersterone	prdnisolone
acetaminophen-hydrocodone	cetirizine	fluticasone	meloxicam	prednisone
acetaminophen-oxycodone	cholestyramine	fluticasone-salmeterol	metformin	pregabalin
acetaminophen-propoxyphene	ciprofloxacin	fluvoxamine	methylphenidate	primidone
acyclovir	citalopram	folic acid	metoclopamide	prochlorperazine
albuterol	clindamycin	furosemide	metoprolol	propoxyphene
alendronate	clomipramine	gemfibrozil	modafinil	propranolol
alendronate-cholecalciferol	clonazepam	glimepiride	mometasone nasal	quetiapine
allopurinol	clopidogrel	glipizide	montelukast	raloxifene
alprazolam	clotrimazole	hydrochlorothiazide	morphine	ranitidine
amiodarone	colesevelam	hydrochlorothiazide-lisinopril	mutivitamin	risedronate
amitriptyline	conjugated estrogens	hydrochlorothiazide-olmesartan	nadolol	ropinirole
amlodipine	cyclobenzaprine	hydrochlorothiazide-triamterene	naproxen	rosiglitazone
amlodipine-benazepril	dexamethasone-tobramycin-ophthalmic	hydrocodone	niacin	rosuvastatin
amoxicillin-clavulanate	dexoxin	hydromorphone	nifedipine	senna
aprepitant	digoxin	hydroxychloroquine	nitroglycerin	sertralinein
aspirin	diltiazem	hydroxyzine	nystatin lidocaine	spironolactone
atenolol	docusate	imipramine	omeprazole	Stoffel's Solution
atenolol-chlorthalidone	domadinol	insulin	oncology swish	sucralfate
atorvastatin	dorzolamide-timolol ophthalmic	iron polysaccharide	ondansetron	sulfamethoxazole-trimethoprim
atropine-diphenoxylate	doxepin	isosorbide mononitrate	oxybutynin	tamsulosin
benazeril	duloxetine	ketoconazole-topical	oxycodone	terazosin
bisoprolol	enalapril	lansoprazole	pantoprazole	timolol-ophthalmic
brimonidine-ophthalmic	envastaurine	latanoprost - ophthalmic	paroxetine	tramadol
budesonide-formoterol	ergocalciferol	letrozole	perindopril	trazodone
bupropion	escitalopram	levetiracetam	phenytoin	triazolam
buspirone	ezetimibe-simvastatin	levofloxacin	Pindolol	valsartan
calcium carbonate	ezitimibe	levothyroxine	pioglitazone	varenicline
carisoprodol	famotidine	lidiphenhyrdramine	PLO OBH	venlafaxine
	felodipine	lisinopril	polyethylene glycol 3350	verapamil
	fenofibrate	lorazepam	potassium chloride	warfarin
	ferrous sulfate	loteprednol-opthalmic	pramipexole	zolpidem
	fexofenadine			
	filgrastim			
	finasteride			

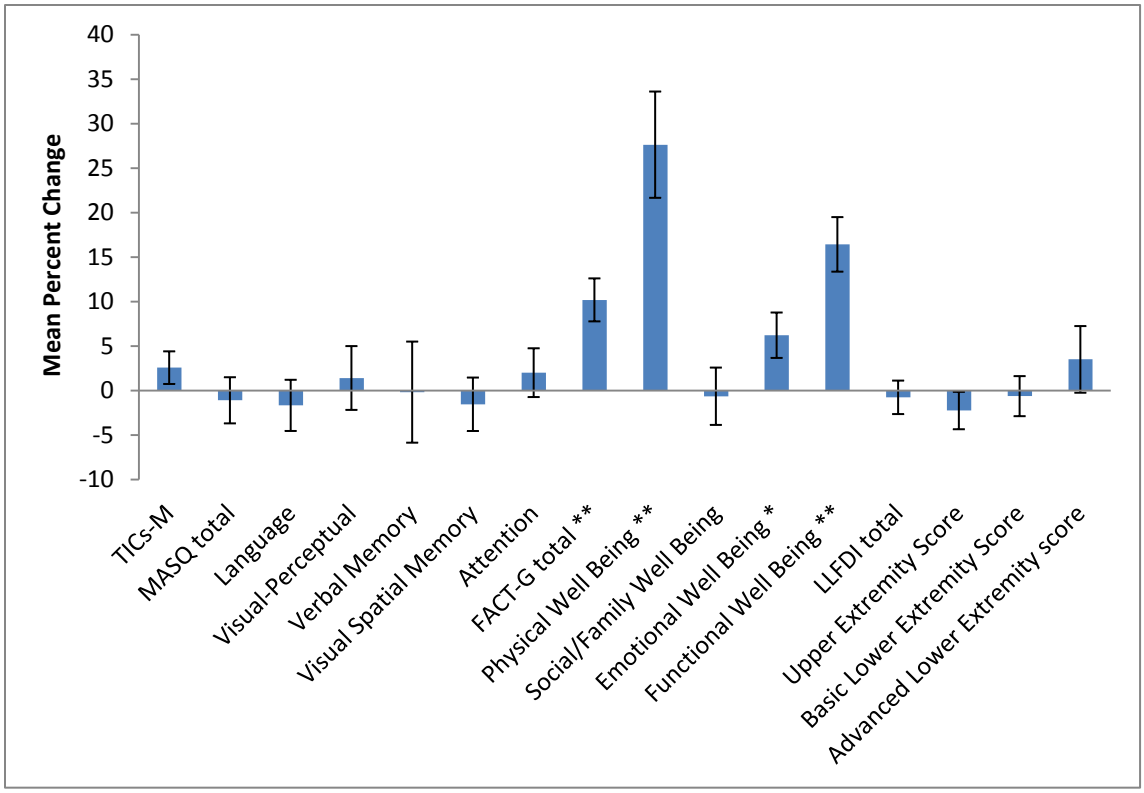

Figure 15 The mean percent change from baseline for cognitive and frailty measures.

Error bars represent standard error

* P value < 0.05, ** P value < 0.0001

CHAPTER 6

CONCLUSIONS AND FUTURE WORK

It is now recognized that cancer and cancer-treatment can lead to declines in cognitive function in a subset of patients. However, the research regarding this phenomenon is relatively new and there are many questions that still need to be further explained. For example we don't know when the deficit begins, the time course and recovery over time, the exact domains, the best method/marker to identify cognitive decline, and the exact mechanisms involved. The ultimate goal is to be able to predict which individuals are at a greater risk of developing cognitive decline in order to target them with treatments. Safety pharmacogenomics, i.e. using genetic variations to predict response/toxicity, offers an exciting approach to identify those patients who are most likely to suffer from cognitive deficits post chemotherapy. Consequently specific therapeutic approaches can be developed to target this group of patients, and/or alternate chemotherapeutic regimens can be used to limit toxicity, thereby offering a way to individualize therapy.

In chapter two we provided a summary of the current status of the literature regarding cognitive decline post chemotherapy. We reviewed the most affected cognitive domains, the possible mechanisms of decline and other confounders, the possible risk factors, and we provided some possible management strategies. And in chapter three we provided an overview of some candidate genes, and the most commonly studied SNPs in relation to cognition. We described what we know about their effect, and what has been done regarding those SNPs in cancer patients. A lot of the information that we have about these candidate SNPs come from studies that involved psychiatric samples.

In our research we studied the effect of 16 SNPs in 6 genes on cognition in a sample of healthy older adults. Our results followed what has been published previously, but also offered some additional information about the roles of those SNPs in cognition.

We found that SNPs that affect serotonin, dopamine and glutamate levels in the brain influence cognition in a healthy sample of older adults possibly in a domain specific manner. Thereby identifying a group of people who inherently have lower cognitive functioning in some domains but that is still within the normal range. In addition individuals with SNPs that previously were associated with lower levels of myeloperoxidase performed better on the executive functions, verbal memory, verbal IQ and IQ. SNPs associated with lower levels were also associated with improvement in self reported verbal and visual memory post chemotherapy. APOE E2 allele was associated with higher cognitive performance compared to other alleles. However we didn't see an effect of APOE post chemotherapy.

APOE E2 allele was associated with higher cognitive performance compared to other alleles which is still in accord to what is known about APOE and Alzheimer's disease. We didn't see an effect of APOE post chemotherapy. Future studies should focus on further understanding the biological roles of those SNPs in cognition, which will help in understanding the pathophysiology of cognitive decline post chemotherapy.

In chapter five, we studied the effects of 31 SNPs in 15 genes on cognition post chemotherapy. Changes were seen in the domains of verbal memory, visual perceptual memory, and attention of the Multiple Ability Self Report Questionnaire, but only when groups were stratified by genotype.

We determined relevant polymorphisms related to cognitive function and frailty from a community dwelling population. Contrary to our hypothesis, patients showed improvements in physical function after chemotherapy and no change in cognitive function. However, using patient stratification based on genotype, specific groups of patients had a measurable decline in cognitive function post chemotherapy. Cognitive declines were detected in the visual spatial memory domain, and attention domain of the MASQ. Interestingly a SNP in the DNA replication enzyme and the target of doxorubicin topoisomerase II was associated with varying degree of self reported attention;

specifically the AA genotype of rs471692 was associated with statistically significant decline in attention post chemotherapy. This indicates that cognitive changes following chemotherapy can be subtle, and stratification by genotype helps us in identifying susceptible individuals and provides some insights on the inconsistencies that are frequently reported in the literature. We also identified genotypes related to cognitive improvement post chemotherapy. For example the TT genotype of Pgp C3435T was associated with a higher rate of improvement in function compared to the CC or CT genotype.

The effect of rs1476455 of GRM3 was in a similar direction in both studies, the AC genotype carriers performing better on numeracy in elderly study and having better emotional well being post chemotherapy. This was also true for P-gp C1236T. In the elderly study presented in chapter 4, the TT genotype performed better on Rey-Osterrieth Complex Figure-Copy as well as numeracy, R-AVLT first learning trial of 15 items, and matrix reasoning of WAIS compared to CC or CT genotypes. And post chemotherapy, the TT genotype didn't have any change from baseline on self reported visual spatial memory as measured by MASQ while the CC genotype had a decline. It is interesting that the TT genotype of C1236T was associated with better performance in visual spatial ability measured objectively in the elderly and self-reported post chemotherapy.

Because of the limitation of the study design we couldn't use the results from the first study as normative data for the second. The patients were not matched, and the instruments used to measure cognition between the two studies were different. However, we studied similar SNPs in two different populations that gave us useful information.

Our study of cognition post chemotherapy is important because it is conducted in a sample of community dwelling lymphoma patients. This population is under-represented in cognition studies, which focused largely on breast cancer patients. We also applied a known method of adjusting for multiple testing in a novel way. The Q value method has been used mainly in studies involving microarrays. We roughly chose a 0.5

limit because our study was exploratory and we didn't want to lose information by being too stringent. But it would have been preferable if previous standards were set. We were limited by sample size, and we had to eliminate some categories in order to have statistically meaningful and generalizable results.

If our results were replicated in larger samples, they will help in defining and managing patients that are most likely to have deficits post chemotherapy, thereby improving their quality of life. They will also provide clues to the biological mechanisms behind cognitive decline, which will lead to identifying drug targets for the management of cognitive decline post chemotherapy, as well as in other cognitive-related diseases.

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