Genetics of Depression Snap Shot

Sites Participating: All sites and all patients in the HALT-C Trial

Principal Investigator: Herb Bonkovsky, MD (University of Connecticut)

Co-Investigators: Henry R. Kranzler, M.D; Amira Pierucci-Lagha, Ph.D.; Jonathan Covault, M.D., Ph.D.

Study Name: GENETIC PREDICTORS OF DEPRESSIVE SYMPTOMS AMONG PATIENTS TREATED WITH INTERFERON-α

Separate Consent Form: No

Withdrawal Form: Yes (Form #9)

Eligible Patients: All Lead-in patients and Express patients who provide consent for genetic testing.

Visit Schedule:
Blood samples are obtained at baseline and during follow-up for the preparation of DNA from patients who provide consent for genetic testing. DNA is prepared by the repository and has already been sent to Dr Bonkovsky for other already ongoing ancillary studies. The leftover DNA will be used for this study.

Genetic Consent:
This consent is part of the main consent form for the HALT-C trial. Genetic consent is recorded on form 4 and may be changed by the patient at any time. The current status of this consent is checked before DNA is prepared or samples are tested.

GENETIC PREDICTORS OF DEPRESSIVE SYMPTOMS AMONG PATIENTS TREATED WITH INTERFERON-α

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A. SPECIFIC AIMS

This study will examine the hypothesis that polymorphisms associated with genes encoding proteins that play a central role in serotonin neurotransmission (e.g., the serotonin transporter protein), and arachidonic acid (AA) metabolism [e.g., fatty acid co-enzyme A ligase 4 (*FACL4*)], moderate the effect of interferon-α (IFN-α) therapy on mood symptoms. This proposal will employ a pharmacogenetic approach to address the following specific aims:

A.1. To determine whether alleles at two candidate genes implicated in susceptibility to depression predict a depressive response among patients treated with IFN-α. We hypothesize that patients who are homozygous for the L allele at a polymorphic site in the promoter region of the gene encoding the serotonin transporter, or who have the intron 1 T-allele variant of *FACL4* will have a significantly greater depressive response to treatment with IFN-α therapy than patients without these genotypes.

A.2. To determine whether these genotypic predictors have an additive or interactive effect on the depressive response to IFN-α treatment. We hypothesize that the effects of these genes will be additive, such that subjects with the risk-associated genotype for both of these candidate genes will have the highest risk of depression during IFN-α treatment.

The ultimate goal of this research is to identify individuals who may be at increased risk for the development of depression secondary to IFN-α therapy and who may benefit from prophylactic antidepressant treatment.

B. BACKGROUND AND SIGNIFICANCE

B.1. Psychoneuroimmunology of depression

The relationship between immune function, psychiatric disorders and the stress response has been the subject of considerable investigation. Activation of the inflammatory response system is thought to be an integral component of the biological response to both acute and chronic stress (Black 2003). Cytokines, which play a key component in the inflammatory response by communicating among cellular components activated by the inflammatory response system have also been shown to produce signaling in the nervous system. In this capacity, cytokines modulate neuroendocrine, cognitive, and behavioral functions (Kronfol and Remick 2000). The most active area of research on CNS effects of cytokines has involved the study of depression and changes in immune function (Licinio and Wong 1999, Smith 1991, van West and Maes 1999). However, it is debated whether alterations in immune function in depression not associated with co-morbid physical illness are an epiphenomenon, the result of depression, or whether they play a pathophysiologic role in depression. Multiple observations suggest interactions of the inflammatory response system and depression: 1) Elevations in pro-inflammatory cytokines have been reported in depression by some (Levine et al. 1999, Maes et al. 1995, Maes et al. 1994), but not all, research groups (Anisman et al. 1999; Haack et al. 1999) and are thought to represent an imbalance involving a relative activation of the monocyte/macrophage arm and relative inhibition of lymphocyte functions. 2) Somatic illness

that frequently has elevated cytokine levels (e.g., rheumatoid arthritis, multiple sclerosis, inflammatory bowel disease, and brain trauma) has high rates of co-morbid depression. A recent study of cancer patients found elevated levels of IL-6 in cancer patients with depression compared with cancer patients compared with depression or healthy controls (Musselman et al. 2001). 3) Depressive symptoms are a common side effect of IFN therapy. Similarly, endotoxin stimulation of inflammatory cytokines in healthy subjects induces an increase in anxiety, depressed mood and a decrement in memory performance in the absence of physical illness (Reichenberg et al. 2001). Studies have shown a clear association between IFN-α treatment and reduced serum tryptophan availability for serotonin biosynthesis and the presence of depressive symptoms in cancer patients (Capuron et al. 2002). Pretreatment with the SSRI paroxetine can prevent the development of major depression in patients undergoing IFN-α therapy (Musselman et al. 2001). 4) Arachidonic acid (AA) and its eicosanoid products are generally pro-inflammatory and are associated with release of pro-inflammatory cytokines, while eicosapentaenoic acid (EPA) and its eicosanoid products compete with AA and its products to produce anti-inflammatory actions (Blok et al. 1996, Cleland and James 2000, Endres and von Schacky 1996, Lands 1992). Studies of AA and EPA levels in depression have shown that the ratio of AA to EPA in cellular membranes correlates with the severity of depressive symptoms (Adams et al. 1996, Maes et al. 1999, Peet et al. 1998). It has been suggested that the beneficial effects of EPA dietary supplements in depression include the ability of EPA to reduce cytokines and other components of inflammatory reactions (Stoll and Locke 2002), which also appears to be the basis for the benefits of omega-3 fatty acid supplements in arthritis, Crohn's disease and atherosclerosis (Belluzzi et al. 1996, Blok et al. 1996, Cleland and James 2000, Curtis et al. 2002, Valagussa et al. 1999). Thus, an excess of AA versus EPA in depression is hypothesized to produce a pro-inflammatory milieu contributing to the pathophysiology of depression.

In this ancillary study of the HALT C Trial, we will further develop the understanding of the psychoneuroimmunology of depression by examining a polymorphism associated with the gene encoding the serotonin transporter (genetic locus *SLC6A4*) and a polymorphism in a gene encoding a protein associated with fatty acid co-enzyme A (genetic locus *FACL4*). These polymorphisms have been associated with functional changes in the enzymatic re-uptake/re-sequestration of serotonin and arachidonic acid, respectively.

B.2. Depression and IFN treatment

IFN-α causes depressive symptoms in patients receiving the medication for the treatment of chronic hepatitis C, multiple sclerosis, or melanoma or other malignancies (Malek-Ahmadi 2001). In fact, depression is the most common severe side effect and the most common reason for IFN-α dose modification or discontinuation (Kraus et al. 2000). A meta-analysis revealed that the mean frequency of depression during a six-month treatment period was 7% with 3 million units (MU) of IFN-α and 10% with doses >5 MU (Poynard et al. 1996). Furthermore, a more recent meta-analysis by the same group (Thevenot et al. 2001) found that patients treated with 3 MU three times per week for more than 6 months had a higher risk of depression (24% vs. 12%; $P < 0.001$) than those treated for a 6-month duration. In carefully controlled studies, the frequency of IFN-α-induced depressive symptoms meeting criteria for a major depressive episode is as high as 33% (Hauser et al. 2002). It has also been reported that the mood symptoms caused by IFN therapy are generally improved by decreasing the dose of IFN-α, and that these effects disappear after cessation of therapy.

B. 3. IFN effects on the serotonin system

The mechanism by which IFN-α induces depressive symptoms in some patients is poorly understood. Evidence of a role of the serotonin system in the production of these symptoms comes from a recent study of cancer patients undergoing IFN-α and/or interleukin-2 therapy. These individuals exhibited significant decreases in serum concentrations of tryptophan (TRP), a serotonin precursor, and in the ratio of TRP to large neutral amino acids (i.e., tyrosine, phenylalanine, leucine, isoleucine, valine), which correlated with increased depression scores (Capuron et al 2002b). The mechanisms by which IFN-α and/or interleukin-2

induce peripheral TRP depletion during cytokine therapy are unknown but may involve activation of the enzyme, indoleamine-2,3-dioxygenase (IDO). IDO is induced by viral infection, lipopolysaccharides, or IFNs, resulting in enhanced catabolism of TRP via the kynurenine (KYN) pathway. Recently, Capuron et al. (2003) tested the hypothesis that TRP degradation into KYN by the enzyme IDO during immune activation may contribute to depressive symptoms during IFN-α therapy. In a study in which 26 patients with malignant melanoma were randomly assigned to receive the SSRI paroxetine or placebo, beginning 2 weeks before IFN-α treatment and continuing for 12 weeks of IFN-α therapy, significant increases in plasma KYN and neopterin, as well as in the KYN/TRP ratio, were observed, irrespective of antidepressant treatment (Capuron et al. 2003). Increases in these compounds have been previously described in patients with chronic hepatitis C undergoing IFN-α therapy (Bonnaccorso et al. 2002, Fischs et al. 1992). Capuron et al. (2003) interpreted their findings to indicate that reduced TRP availability plays a role in IFN-α-induced depressive symptoms, and that paroxetine, although not altering the KYN or neopterin response to IFN-α, attenuates the behavioral consequences of IFN-α-mediated TRP depletion. Further support for the role of serotonin in IFN-induced depressive symptoms is provided by experiments in which recombinant human IFN-α-2a attenuated serotonin type 2 receptor function and murine IFN-α increased levels of serotonin transporter mRNA (Morikawa et al. 1998).

B. 4. Candidate genes related to depression in IFN treatment and modulation of serotonin signaling: The serotonin transporter

Because depression induced by IFN-□ can be treated with selective serotonin reuptake inhibitors (SSRI's) such as fluoxetine or paroxetine (Levenson et al. 1993, Hauser et al.), the serotonin transporter (5-HTT) protein, which is a major site of action of these medications, may play a role in the pathophysiology of IFN-induced depressive symptoms. 5-HTT, which is involved in the presynaptic reuptake of serotonin to terminate serotonergic neurotransmission, has been implicated in the etiology of depression. The gene encoding 5-HTT has been localized to chromosome 17q11.2. The promoter region of the gene is characterized by an insertion-deletion polymorphism (*5-HTTLPR*), which produces a short ("S") allele, which is associated with lower transcriptional efficiency of the promoter than the long ("L") allele (Lesch et al. 1996). In human placental choriocarcinoma (BeWo) cells, Morikawa et al. (1998) found that 3 hours of treatment with IFN-□ or IFN-□increased levels of 5-HTT mRNA, an effect that was inhibited by treatment with actinomycin D, an inhibitor of transcription. Treatment with IFN-□ or IFN-□for 3-6 h, but not for 30 min, also increased 5-HTT uptake activity. These data are consistent with the hypothesis that IFN-induced psychiatric side effects arise through regulation of 5-HTT transcription, providing support for an etiological role of this mechanism in IFN-induced affective disorders.

Rapid depletion of serum tryptophan (RTD) has been widely used to study central 5-HT function in major depressive disorder (MDD). Depletion of tryptophan produces a transient decrease in brain 5-HT levels, resulting in a transient increase in depressive symptoms among remitted depressives. Moreno et al. (2002) found an association between *5-HTTLPR* alleles and the depressive response to RTD in treated depressed subjects, with the LL genotype being associated with a significantly lower mood after RTD than the LS or SS genotype. The finding that depressive disorder patients with the LL genotype may be at increased risk of relapse to a depressive episode following antidepressant treatment was recently replicated in a sample of depressed alcoholics (Pierucci-Lagha et al., in press). In addition, the LL genotype has been associated with an earlier and greater response to SSRI therapy for major depression than that observed in individuals with the LS or SS genotype (Smeraldi et al. 1998, Benedetti et al. 1999, Zanardi et al. 2000, Pollock et al. 2000, Yu et al. 2002, Rausch et al. 2002).

5-HTTLPR genotype also appears to have a significant interactive effect with stressful life events in the production of depressive symptoms. In a birth cohort study of 1037 subjects, the association of the *5-HTTLPR* S allele and depression was moderated by the number of prior exposures to stressful life events, both during developmental and adult periods (Caspi et al. 2003). The *5-HTTLPR* genotype was unrelated to risk of depression in subjects without prior

adult stressful life events (or childhood maltreatment). In subjects exposed to either childhood maltreatment or to adult life stressors, subjects with the SS genotype had a doubling of risk for major depressive disorder by age 26 compared with the LL genotype, whereas heterozygote subjects had an intermediate risk. In a study using the tryptophan depletion method, healthy women with the *5-HTTLPR* S allele had increased depressive symptoms following tryptophan depletion in a gene-dose dependent fashion (Neumeister et al. 2002).

B.5. Candidate genes related to depression during IFN treatment and modulation of arachidonic acid signaling: Fatty acid co-enzyme A ligase type-4 (*FACL4*)

FACL4 is a key enzyme involved in the metabolism of arachidonic acid (AA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and it is highly expressed by neurons of the developing and adult brain (Cao et al. 1998; Piccini et al. 1998). FACL4 selectively esterifies these fatty acids with co-enzyme A, forming acyl-co-A, which can then be incorporated into membrane phospholipid. FACL4 is thought to regulate the availability of AA and EPA as precursors for the generation of eicosanoids following stimulus-coupled release of AA and EPA from membrane phospholipids (Cao et al. 1998; Wilson et al. 1982). Free AA and EPA are maintained at very low levels by their esterification by FACL4 with co-enzyme A. Maintenance of low basal levels of AA and EPA limits the production of bioactive eicosanoids from AA and EPA to periods of agonist-induced release of AA and EPA from membrane stores. We have recently identified a C to T SNP in intron 1 of *FACL4* that is associated with depression (Covault et al. 2003) and appears to be linked with a functional polymorphism of *FACL4* that reduces enzyme activity. The cellular processes by which reduced FACL4 activity may contribute to the risk of depression are unclear, but may relate to the hypothesized role of elevated inflammatory cytokines in depression. The negative effect of an excess of AA relative to EPA in depression would likely be aggravated by a reduced rate of FACL4 re-sequestration of agonist-released AA. Finally, abnormal regulation of free AA levels by a hypoactive FACL4 could lead to enhanced apoptosis in the developing nervous system or following metabolic or environmental stress as higher levels of free AA generated by inhibition of FACL4 promote apoptosis in model systems (Cao et al. 2000).

C. PRELIMINARY STUDIES.

C.1. Effects of Rapid Tryptophan Depletion (RTD) on Mood and Urge to Drink in Patients with Co-Morbid Major Depression and Alcohol Dependence (Pierucci et al., in press)

We conducted a preliminary study of the effects of RTD on mood and urge for alcohol in alcohol-dependent patients with co-morbid major depression (Pierucci et al., in press). Based, on our finding of an association of *5-HTTLPR* alleles with co-morbid major depressive disorder (Nellissery et al. 2003) and alcohol dependence, we also examined the moderating effect of this genotype on response to RTD.

Methods: Fourteen subjects (50% male) aged 21-59, who met criteria for current alcohol dependence and major depression completed this double blind, placebo-controlled study. Half of the participants were among those who showed a response to treatment during a 10-week placebo-controlled study of nefazodone and the other half responded to treatment while participating in a 10-week placebo-controlled trial of sertraline. The criteria for a treatment response was a 50% reduction in the Hamilton Depression Rating Scale (HDRS) score or an absolute score of less than 8 on the Hamilton Rating Scale for Depression (HAM-D), and a 50% reduction of their baseline drinking.

Following the treatment trial and while still on study medication, patients underwent two day-long experimental sessions, 7-10 days apart. For the 24 hours prior to each session, patients consumed a TRP-deficient diet. During the first session, patients ingested a TRP-depleting amino acid mixture. During the second session, a similar mixture of amino acids was ingested; the only difference being that TRP was included. During each session mood was measured at regular intervals. Mood measures included the Hamilton Rating Scale for

Depression (HAM-D), the Beck Depression Inventory (BDI), and the Spielberger State-Trait Anxiety Inventory (STAI).

Results: Five hours after the TRP depletion session, plasma TRP levels decreased by 73.1%, whereas during the sham session TRP levels increased by 32.4 % [Time X session: $F(1,13) = 74.10$, $p<.001$]. Although RTD did not increase depressive symptoms over baseline for the group as a whole, HAM-D scores decreased during the sham session, resulting in a significant main effect of session [$F(1,13) = 12.32$, $p=.004$]. When a criterion of $\geq$ 50% increase in HAM-D score was used as a measure of depressive symptom exacerbation, half of the subjects showed a depressive effect of RTD (FET: $p=.003$). Depressive exacerbation was more evident in antidepressant responders compared to placebo responders [Pillai's $F(3,10) = 4.59$, $p=.029$].

When data were analyzed in relation to *5-HTTLPR* genotype, there was a significant interaction of genotype X session both on BDI and HAM-D scores [$F(1,11) = 5.71$, $p=.036$ and $F(1,11) = 5.16$, $p=.044$, respectively] (Figures 1A and 1B). During RTD, individuals homozygous for the L allele reported greater depression than did subjects carrying the S allele.

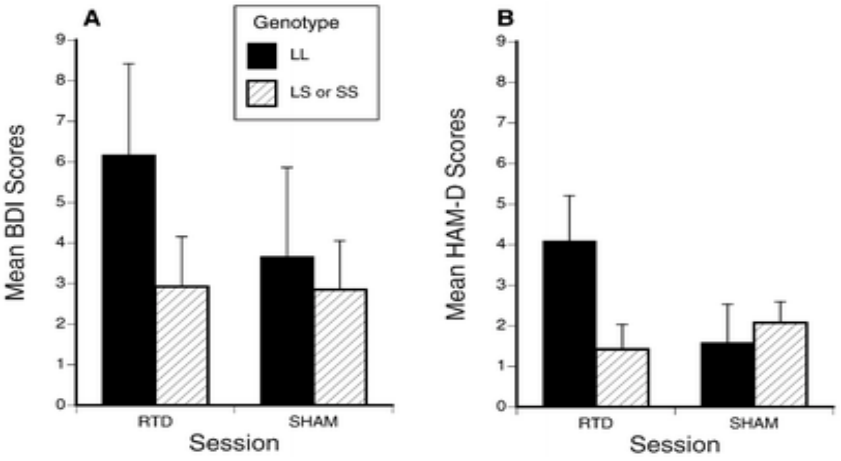

Figure 1A. Mean (SEM) BDI score by session and genotype: *BDI* Beck depression inventory, *RTD* rapid tryptophan depletion, *LL* homozygous for the long allele, *LS* or *SS* heterozygous or homozygous for the short allele.

Figure 1B. Mean (SEM) HAM-D scores by session and genotype: *HAM-D* Hamilton depression rating Scale

Discussion: This pilot study provides preliminary support for the use of the RTD paradigm to assess mood and the urge for alcohol in depressed alcoholics. It also supports the utility of RTD response as a phenotype for genetic analysis, the ultimate goal of which is to identify individuals who may be at increased risk for co-morbid major depression and alcohol dependence, to predict the response to pharmacotherapy and to better understand the process of relapse to major depression and/or alcohol dependence.

C.2. Association of an *FACL4* Polymorphism with Depression and with Enhanced Niacin-Induced Dermal Erythema (Covault et al., in press)

Using a case-control study design, we examined the allelic association of *FACL4* (long-chain fatty acid-CoA ligase type 4) with depression and schizophrenia, the two disorders for which arachidonic acid (AA) metabolism may be of pathophysiologic importance (Covault et al.; in press). FACL4 is a key enzyme involved in the metabolism of AA. Conversely, a functional change in an *FACL4* exon or genetic control element, resulting in increased FACL4 activity and more rapid re-sequestration of free AA, would be associated with a reduced niacin-induced dermal erythema (as observed in many subjects with schizophrenia).

Methods: The niacin-stimulated release of AA from skin was used as a biomarker to identify a common polymorphism associated with *FACL4,* which was correlated with niacin induced erythema. We examined the allelic frequency of this polymorphism in 555 European Americans, including 229 control subjects, 198 subjects with major depression, 58 with schizophrenia or schizoaffective disorder, and 70 with alcohol dependence without co-morbid psychiatric illness.

***Results**:* We observed that a common C to T polymorphism in the first intron of *FACL4* is associated with the degree of redness in the niacin-induced dermal erythema test. Male subjects (control or schizophrenia) with the T0 genotype have an increased dermal erythema response to topical niacin as compared with their C0 genotype. This suggests that this polymorphism may be in linkage disequilibrium with a functional polymorphism of the FACL4 gene that modulates re-sequestration of agonist-released free AA. We also observed a significant excess of the T allele in subjects with major depression, as compared with controls (49% vs. 38%; p=0.003) and a non-significant excess of the T allele in schizophrenia (44%; p=0.29). The allele frequency for subjects with alcohol dependence did not differ from controls.

Conclusion: This is the first examination of an association of *FACL4* alleles to any psychiatric disorder or associated endophenotype. We observed an increased frequency of a common single nucleotide polymorphism associated with the FACL4 gene in major depression, as well as an increase in dermal erythema in male control and schizophrenia subjects having this variant. The results are consistent with linkage of this SNP to a functional change that reduces FACL4 enzyme activity, which may contribute to the complex pathophysiology of depression.

D. DESCRIPTION OF PROPOSED RESEARCH

D.1. Overview: This study will employ a pharmacogenetic approach to understand the pathophysiology of depressive symptoms among patients undergoing IFN-α therapy. We propose to test the stated hypotheses by analysis of data and blood samples that have already been obtained from subjects enrolled in the HALT C Trial. The ultimate goal of this research is to identify individuals who may be at increased risk to develop depression secondary to IFN-α therapy and who may benefit from prophylactic antidepressant treatment. This will make it possible to prevent depression among susceptible individuals, thereby enhancing the potential therapeutic effects of IFN-α therapy without subjecting individuals who are not predisposed to depression to unnecessary antidepressant prophylaxis. The availability of pretreatment data on psychiatric diagnoses and longitudinal data on depressive symptoms during treatment, as well as DNA for genotyping the *5-HTTLPR* and *FACL4* polymorphisms, will make it possible to test the stated hypotheses.

D.2. Patients: All individuals for whom DNA is available from the Phase I sample of the HALT C Trial will be genotyped for the candidate genes. Blood samples drawn at baseline from all participants enrolled at all sites will be used for this study.

D.3. Clinical assessments required for this proposal:

Data from the *Beck Depression Inventory* (BDI; Beck et al. 1961) will be used as a measure of depressive symptoms.

Data from the *Composite International Diagnostic Interview* [CIDI-Auto (version 2.1): WHO 1993], a computerized, self-administered interview for the assessment of mental disorders will be used to provide psychiatric diagnoses, including the following diagnoses: anxiety diagnosis, depression diagnosis, alcohol abuse or dependence diagnosis, and drug abuse or dependence diagnosis.

D.4. Genotyping*:* Genotyping will all be carried out by the UCHC General Clinical Research Center under the direction of Dr. Covault. Samples of DNA isolated from peripheral blood samples by BBI, the specimen reposition of the HALT C Trial, have already been sent to Dr Bonkovsky and our core Lab where genetic variations of several other genes being determined for other already ongoing ancillary studies (Role of iron, HFE, and other genetic factors). We will use “left-over” DNA for the studies proposed herein. We do not anticipate requiring any additional DNA.

5-HTTLPR: Genotyping for the *5-HTTLPR* insertion/deletion polymorphism will be carried out by analysis of PCR amplicon length using agarose gel electrophoresis (Gelernter et al. 1997). 10 ng of genomic DNA will be in the presence of 1M betaine using primers ATGCCAGCACCTAACCCCTAATGT and amplified GGACCGCAAGGTGGGCGGGA for 30 cycles of three temperatures PCR at 98/60/72°C each for 30 seconds. PCR products will be

examined on 2% agarose gels to distinguish the 419-bp long (L) allele of 16-repeat product vs. the 375-bp short (S) 14-repeat allele.

FACL4: A 64 nt fragment encompassing the rs1324805 SNP in *FACL4* intron 1 will be amplified by 33 three-stage PCR thermocycles using 10 ng DNA, 2 mM $MgCl_2$, primers 5'-TGATTTTCAGCGCGAGGAGT and 5'-GGATTCAAGGCAGCTAGCAGG with an annealing temperature of 60° C. PCR products are digested with Hinc II and melting profile determined using an ABI 7700 instrument (Applied Biosystems, Foster City, CA) following the addition of 3 volumes of 10% DMSO, 20 mM NaCl, 1x SybrGreen (Molecular Probes, Eugene Oregon). The C allele is cut by HincII, producing a product with Tm=76° C vs. Tm=83° C for the undigested T allele PCR product.

D.5. Analytic plan: We hypothesize that among IFN-α-treated patients, those who are homozygous for the L allele at a polymorphic site in the promoter region of the gene encoding the serotonin transporter (*5-HTTLPR*), or the intron 1 T-allele variant of *FACL4* will show a significantly greater increase in depressive symptoms during treatment with IFN-α therapy. We also hypothesize that the effects of these genes will be additive, such that subjects with the risk-associated genotype for both of these candidate genes will have the highest risk of depression during IFN treatment.

An important issue preceding evaluation of outcomes will be to identify baseline differences between groups that may have occurred despite the use of urn randomization. The distribution of data will be examined prior to analysis, to determine the need for transformation and whether parametric analytic methods can be utilized.

We will first examine the frequency and correlates of lifetime and current major depression and their association with alleles of the *5-HTTLPR* and *FACL4* polymorphisms. We will then use hierarchical linear modeling to examine the relations between genotype for each of the two genes (a three-level variable for *5-HTTLPR* and a two-level (men) or three-level (women) variable for *FACL4*) and scores on the Beck Depression Inventory (BDI), with depression diagnosis (a three-level variable: never, past, current) as a factor in the analysis. It should also be noted that hierarchical linear modeling, in contrast to repeated measures ANOVA, for example, is robust to missing observations and to variation in the interval between repeated measures. Given that *FACL4* is X-linked, we will conduct analyses of the allelic association of this gene separately for men and women.

For the analyses, we will use data from phase I of the HALT C Trial, during which subjects received high doses of IFN-α + ribavirin for 24 weeks. The time points for the analysis will be at 0, 12 and 24 weeks of treatment. In addition to examining the time X genotype interaction, we will also examine the time X genotype X depression diagnosis and gene-gene interactions. The impact of sex, race, and treatment site will be examined in all analyses and, if significant, will be retained in the final equations. Since some patients will have received antidepressant treatment, we will evaluate that measure as a time-varying covariate in the analyses.

D.5.1. Sample size estimation:

We estimated the sample size required to examine the moderating effect of the two polymorphisms on depression risk, based on preliminary findings from the HALT C trial. To do this, we used data indicating that approximately 35% of 1023 HALT C participants reported a change in their depression status from baseline to endpoint, including 14.1% who changed from depressed to not depressed and 20.6% who changed from not depressed to depressed. The dependent samples McNemar change test showed a medium effect of treatment on depression status [odds ratio = 2.12 (95% CI = 1.70, 2.52); $\square^2 = 23.3$, 1 df, $p < .001$].

Assuming Hardy-Weinberg equilibrium in the distribution of *5-HTTLPR* genotypes, with a frequency of 0.6 for the L allele and 0.4 for the S allele, 33.3% of participants (n = 334) will be homozygous for the L allele and 66.7% of participants (n = 687) will be heterozygous or homozygous for the S allele. Setting $\square$ equal to .05 for a directional hypothesis, this sample will provide power = .80 if the odds ratio for the genotype groups is 1.76 (i.e., a small effect, which corresponds to a difference of 11.8% between the genotype groups in the number of

participants who went from not depressed to depressed). Assuming an odds ratio of 2.0 (i.e., a small-to-medium effect, which corresponds to a 14% difference between genotype groups in the number of participants who went from not depressed to depressed), this sample will provide power = .91.

Because *FACL4* is X-linked, it will be necessary to examine the allelic association with depression induced by IFN-□ separately for men and women. We estimated statistical power to detect an effect for men, because they comprise 70% of the sample. The association of the T allele at FACL4 with depression yielded an OR of 1.92 (Covault et al., in press). Based on this estimate, □ equal to 0.05 for a directional hypothesis, the power provided by this sample to detect a statistically significant effect is 0.91.

Based on these estimates, we are confident that, if an effect of either *5-HTTLPR* or *FACL4* genotype on risk of INF*a*-induced depression exists, we will be able to detect it.

E. HUMAN SUBJECTS

E.1. Characteristics of subject population: Subjects will include all individuals that have already participated in the HALT C Trial. They will comprise both sexes, and be of varied ethnic backgrounds.

E.2. Sources of research material: Questionnaires and blood specimens, all obtained exclusively for research purposes.

E.3. Subject recruitment: We are not going to recruit additional subjects for this proposal. Only individuals for whom we have pretreatment data on psychiatric diagnoses and longitudinal data on depressive symptoms during treatment, as well as blood samples drawn at baseline will be used for this study.

E.4. Risk: Since loss of confidentiality is a potential hazard of any protocol involving genetic studies, patients' names won't appear and they will be identified by a coding number. All study data forms will contain only the patient's unique study identification number, using a reference system maintained by the NERI.

E.5. Benefit: There will be no benefit to subjects from indirectly participating in this research proposal.

E.6. Risk-benefit consideration: Given the minimal risk, we believe that the potential benefits of the proposed research, namely, identification of future patients at risk of developing mood disorders after IFN-α therapy, outweigh the risk event of our understanding of the psychoneuroimmunology of depression, and by improvement in knowledge of quantitative predictors of risk. Improved accuracy of risk assessment will make it possible to prevent depression among susceptible individuals, thereby enhancing the potential therapeutic effects of IFN-α therapy without subjecting individuals who are not predisposed to depression to unnecessary antidepressant prophylaxis, which carries with it unwanted adverse effects and costs.

F. BUDGETARY CONSIDERATIONS

The cost of reagents (approximately $1.00 for each measurement) and the costs of technician time and core Lab facilities will be covered by UCHC Institutional funds. The costs of NERI staff time and effort to extract and forward information from the HALT C Trial database will be covered by the support already provided to NERI from the HALT C main Trial budget, and other funded ancillary studies.

G. REFERENCES

Adams P, Lawson S, Sanigorski A, Sinclair A (1996) Arachidonic acid to eicosapentaenoic acid ratio in blood correlates positively with clinical symptoms of depression. *Lipids* 31:S157-161

Anisman H, Ravindran AV, Griffiths J, Merali Z (1999) Endocrine and cytokine correlates of major depression and dysthymia with typical or atypical features. *Mol Psychiatry* 4:182-188.

Beck AT, Ward CH, Mendelson M, Mock J, Erbaugh J. An inventory for measuring depression. *Arch Gen Psychiatry* 4:461-471, 1961.

Belluzzi A, Brignola C, Campieri M, Pera A, Boschi S, Miglioli M (1996) Effect of an enteric-coated fish-oil preparation on relapses in Crohn's disease. *N Engl J Med* 334:1557-1560.

Benedetti F, Serretti A, Colombo C, Campori E, Barbini B, di Bella D, Smeraldi E. Influence of a functional polymorphism within the promoter of the serotonin transporter gene on the effects of total sleep deprivation in bipolar depression. *Am J Psychiatry* 156:1450-1452, 1999.

Black PH (2003) The inflammatory response is an integral part of the stress response: Implications for atherosclerosis, insulin resistance, type II diabetes and metabolic syndrome X. *Brain Behav Immun* 17:350-364.

Blok WL, Katan MB, van der Meer JW (1996) Modulation of inflammation and cytokine production by dietary (n-3) fatty acids. *J Nutr* 126:1515-1533.

Bonaccorso S, Marino V, Puzella VA, Pasquini M, Biondi M, Artini M *et al.*, Increased depressive ratings in patients with hepatitis C receiving interferon-alpha-based immunotherapy are related to interferon-alpha-induced changes in the serotonergic system. *J Clin Psychopharmacol* 22:86–90, 2002.

Cao Y, Pearman AT, Zimmerman GA, McIntyre TM, Prescott SM (2000) Intracellular unesterified arachidonic acid signals apoptosis. *Proc Natl Acad Sci U S A* 97:11280-11285.

Cao Y, Traer E, Zimmerman GA, McIntyre TM, Prescott SM (1998) Cloning, expression, and chromosomal localization of human long-chain fatty acid-CoA ligase 4 (FACL4). *Genomics* 49:327-330.

Capuron L, Gumnick JF, Musselman DL, Lawson DH, Reemsnyder A, Nemeroff CB and Miller AH. Neurobehavioral effects of interferon-alpha in cancer patients: Phenomenology and paroxetine responsiveness of symptom dimensions. *Neuropsychopharmacology* 26:643–652, 2002a.

Capuron L, Ravaud A, Neveu PJ, Miller AH, Maes M and Dantzer R, Association between decreased serum tryptophan concentrations and depressive symptoms in cancer patients undergoing cytokine therapy. *Mol Psychiatry* **7**: 468–473, 2002b.

Capuron L, Neurauter G, Musselman DL, Lawson DH, Nemeroff CB, Fuchs D, Miller AH. Interferon-alpha-induced changes in tryptophan metabolism. Relationship to depression and paroxetine treatment. *Biol Psychiatry* 54:906-914, 2003.

Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, McClay J, Mill J, Martin J, Braithwaite A, Poulton R. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 301:386-389, 2003.

Cleland LG, James MJ (2000) Fish oil and rheumatoid arthritis: antiinflammatory and collateral health benefits. *J Rheumatol* 27:2305-2307.

Covault J, Pettinati H, Moak D, Mueller T, Kranzler K (in press) Association of a long-chain fatty acid-CoA ligase 4 gene polymorphism with depression and with enhanced niacin-induced dermal erythema. *Am J Med Genetics*.

Curtis CL, Rees SG, Little CB, Flannery CR, Hughes CE, Wilson C, Dent CM, et al (2002) Pathologic indicators of degradation and inflammation in human osteoarthritic cartilage are abrogated by exposure to n-3 fatty acids. *Arthritis Rheum* 46:1544-1553.

Endres S, von Schacky C (1996) n-3 polyunsaturated fatty acids and human cytokine synthesis. *Curr Opin Lipidol* 7:48-52.

Fuchs D, Norkrans G, Wejstal R, Reibnegger G, Weiss G, Weiland O *et al.*, Changes of serum neopterin, beta 2-microglobulin and interferon-gamma in patients with chronic hepatitis C treated with interferon-alpha 2b. *Eur J Med* 1:196–200, 1992.

Haack M, Hinze-Selch D, Fenzel T, Kraus T, Kuhn M, Schuld A, Pollmacher T (1999) Plasma levels of cytokines and soluble cytokine receptors in psychiatric patients upon hospital admission: effects of confounding factors and diagnosis. *J Psychiatr Res* 33:407-418.

Hauser P, Khosla J, Aurora H, Laurin J, Kling MA, Hill J, Gulati M, Thornton AJ, Schultz RL, Valentine AD, Meyers CA, Howell CD. A prospective study of the incidence and open-label treatment of interferon-induced major depressive disorder in patients with hepatitis C. *Mol Psychiatry* 7:942-947, 2002.

Hauser P, Soler R, Reed S, Kane R, Gulati M, Khosla J, Kling MA, Valentine AD, Meyers CA. Prophylactic treatment of depression induced by interferon-alpha. *Psychosomatics* 41:439-441, 2000.

Jonsson EG, Goldman D, Spurlock G, Gustavsson JP, Nielsen DA, Linnoila M, Owen MJ, et al (1997) Tryptophan hydroxylase and catechol-O-methyltransferase gene polymorphisms: relationships to monoamine metabolite concentrations in CSF of healthy volunteers. *Eur Arch Psychiatry Clin Neurosci* 247:297-302.

Kraus MR, Schafer A, Faller H, Csef H, Scheurlen M. Paroxetine for the treatment of interferon-alpha-induced depression in chronic hepatitis C. *Aliment Pharmacol Ther.* 16:1091-1099, 2002.

Kraus MR, Wilms K (2000). Interferon alpha. Effect, indications, therapy monitoring and side-effects. Internist (Berl). 41:1399-1404.

Kronfol Z, Remick DG (2000) Cytokines and the brain: implications for clinical psychiatry. *Am J Psychiatry* 157:683-94.

Lands WE (1992) Biochemistry and physiology of n-3 fatty acids. *Faseb J* 6:2530-2536.

Levine J, Barak Y, Chengappa KN, Rapoport A, Rebey M, Barak V (1999) Cerebrospinal cytokine levels in patients with acute depression. *Neuropsychobiology* 40:171-6.

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, Benjamin J, Muller CR, Hamer DH, Murphy DL (1996). Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* 247: 1527-1531.

Levenson JL, Fallon HJ (1993). Fluoxetine treatment of depression caused by interferon-alpha. *Am J Gastroenterol* 88:760-761.

Licinio J, Wong ML (1999) The role of inflammatory mediators in the biology of major depression: central nervous system cytokines modulate the biological substrate of depressive symptoms, regulate stress-responsive systems, and contribute to neurotoxicity and neuroprotection. *Mol Psychiatry* 4:317-327.

Maes M, Christophe A, Delanghe J, Altamura C, Neels H, Meltzer HY (1999) Lowered omega3 polyunsaturated fatty acids in serum phospholipids and cholesteryl esters of depressed patients. *Psychiatry Res* 85:275-291.

Maes M, Meltzer HY, Bosmans E, Bergmans R, Vandoolaeghe E, Ranjan R, Desnyder R (1995) Increased plasma concentrations of interleukin-6, soluble interleukin-6, soluble interleukin-2 and transferrin receptor in major depression. *J Affect Disord* 34:301-309.

Maes M, Scharpe S, Meltzer HY, Okayli G, Bosmans E, D'Hondt P, Vanden Bossche BV, et al (1994) Increased neopterin and interferon-gamma secretion and lower availability of L-tryptophan in major depression: further evidence for an immune response. *Psychiatry Res* 54:143-160.

Malek-Ahmadi P. Mood disorders associated with interferon treatment: theoretical and practical considerations. *Ann Pharmacother* 35:489-495, 2001.

Moreno FA, Rowe DC, Kaiser B, Chase D, Michaels T, Gelernter J, Delgado PL. Association between a serotonin transporter promoter region polymorphism and mood response during tryptophan depletion. *Mol Psychiatry* 7:213-216, 2002.

Morikawa O, Sakai N, Obara H, Saito N. Effects of interferon-alpha, interferon-gamma and cAMP on the transcriptional regulation of the serotonin transporter. *Eur J Pharmacol* 349:317-324, 1998.

Musselman DL, Lawson DH, Gumnick JF, Manatunga AK, Penna S, Goodkin RS *et al.*, Paroxetine for the prevention of depression induced by high-dose interferon alfa. *N Engl J Med.* 344:961-966, 2001.

Musselman DL, Miller AH, Porter MR, Manatunga A, Gao F, Penna S, Pearce BD, *et al* (2001) Higher than normal plasma interleukin-6 concentrations in cancer patients with depression: preliminary findings. *Am J Psychiatry* 158:1252-1257.

Nellissery M, Feinn RS, Covault J, Gelernter J, Anton RF, Pettinati H, Moak D, Mueller T, Kranzler HR (2003) Alleles of a functional serotonin transporter promoter polymorphism are associated with major depression in alcoholics. *Alcohol Clin Exp Res.* 27:1402-1408.

Neumeister A, Konstantinidis A, Stastny J, Schwarz MJ, Vitouch O, Willeit M, Praschak-Rieder N, et al (2002) Association between serotonin transporter gene promoter polymorphism (5HTTLPR) and behavioral responses to tryptophan depletion in healthy women with and without family history of depression. *Arch Gen Psychiatry* 59:613-620.

Peet M, Murphy B, Shay J, Horrobin D (1998) Depletion of omega-3 fatty acid levels in red blood cell membranes of depressive patients. *Biol Psychiatry* 43:315-319.

Piccini M, Vitelli F, Bruttini M, Pober BR, Jonsson JJ, Villanova M, Zollo M, et al (1998) FACL4, a new gene encoding long-chain acyl-CoA synthetase 4, is deleted in a family with Alport syndrome, elliptocytosis, and mental retardation. *Genomics* 47:350-358.

Pierucci-Lagha A, Feinn R, Modesto-Lowe V, Swift R, Nellissery M, Covault J, and Kranzler HR. Effects of rapid tryptophan depletion on mood and urge to drink in patients with co-morbid major depression and alcohol dependence. *Psychopharmacology* 171:340-348, 2004.

Pollock BG, Ferrell RE, Mulsant BH, Mazumdar S, Miller M, Sweet RA, Davis S, Kirshner MA, Houck PR, Stack JA, Reynolds CF, Kupfer DJ. Allelic variation in the serotonin transporter promoter affects onset of paroxetine treatment response in late-life depression. *Neuropsychopharmacology* 23(5):587-590, 2000.

Poynard T, Leroy V, Cohard M, Thevenot T, Mathurin P, Opolon P, Zarski JP. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C: effects of dose and duration. *Hepatology* 24(4):778-789, 1996.

Rausch JL, Johnson ME, Fei YJ, Li JQ, Shendarkar N, Hobby HM, Ganapathy V, Leibach FH. Initial conditions of serotonin transporter kinetics and genotype: influence on SSRI treatment trial outcome. *Biol Psychiatry* 51(9):723-732, 2002.

Reichenberg A, Yirmiya R, Schuld A, Kraus T, Haack M, Morag A, Pollmacher T (2001) Cytokine-associated emotional and cognitive disturbances in humans. *Arch Gen Psychiatry* 58:445-452.

Serretti A, Zanardi R, Rossini D, Cusin C, Lilli R, Smeraldi E (2001) Influence of tryptophan hydroxylase and serotonin transporter genes on fluvoxamine antidepressant activity. *Mol Psychiatry* 6:586-592.

Smeraldi E, Zanardi R, Benedetti F, Di Bella D, Perez J, Catalano M. Polymorphism within the promoter of the serotonin transporter gene and antidepressant efficacy of fluvoxamine. *Mol Psychiatry* 3(6):508-511, 1998.

Smith RS (1991) The macrophage theory of depression. *Med Hypotheses* 35:298-306.

Stoll A, Locke C (2002) Omega-3 fatty acids in mood disorders. In: Mischoulon D, Rosenbaum J (eds) Natural Medications for Psychiatric Disorders. Lippincott Williams & Wilkins, Philadelphia, pp 13-34.

Thevenot T, Regimbeau C, Ratziu V, Leroy V, Opolon P, Poynard T. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C in naive patients: 1999 update. J Viral Hepat. 2001 8:48-62.

Valagussa F, Franzosi M, Geraci E (1999) Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-prevenzione trial. *Lancet* 354:447-455

van West D, Maes M (1999) Activation of the inflammatory response system: A new look at the etiopathogenesis of major depression. *Neuroendocrinol Lett* 20:11-17.

Wilson DB, Prescott SM, Majerus PW (1982) Discovery of an arachidonoyl coenzyme A synthetase in human platelets. *J Biol Chem* 257:3510-3515.

World Health Organization: Composite International Diagnostic Interview, Version 2.1. Geneva, World Health Organization, 1993.

Yu YW, Tsai SJ, Chen TJ, Lin CH, Hong CJ. Association study of the serotonin transporter promoter polymorphism and symptomatology and antidepressant response in major depressive disorders. *Mol Psychiatry* 7:1115-1119, 2002.

Zanardi R, Benedetti F, Di Bella D, Catalano M, Smeraldi E. Efficacy of paroxetine in depression is influenced by a functional polymorphism within the promoter of the serotonin transporter gene. *J Clin Psychopharmacol.* 20:105-107, 2000.