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Proton Magnetic Resonance Spectroscopy in Alcohol Use Disorders: A Potential New Endophenotype?

Dieter J. Meyerhoff and Timothy C. Durazzo

From the University of California San Francisco, VA Medical Center San Francisco, Center for Imaging of Neurodegenerative Diseases, San Francisco, California

Abstract

Background—Current effort is directed at defining new classification schemes for alcohol use disorders (AUD) based on genetic/biological, physiological, and behavioral endophenotypes.

Methods—We describe briefly findings of in vivo brain proton magnetic resonance spectroscopy (^1H MRS) studies in AUD and propose that they be further explored and expanded regarding their value as a potential endophenotype for AUD.

Results—In vivo ^1H MRS, as part of the emerging field of “imaging genomics,” may provide readily accessible, objective, functionally significant and region-specific neurobiological measures that successfully link genotypes to neurocognition and to psychiatric symptomatology in relatively small patient cohorts. We discuss several functional gene variants that may affect specific ^1H MRS-detectable metabolites and provide recent data from our own work that supports the view of genetic effects on metabolite measures.

Conclusions—MRS-genetics research will not only offer clues to the functional significance and downstream effects of genetic differences in AUD, but, via monitoring and/or predicting the efficacy of pharmacological and behavioral interventions as a function of genotype, has the potential to influence future clinical management of AUD.

Keywords

Brain; Neuroimaging; Magnetic Resonance Spectroscopy; Genetics; Endophenotype

Alcohol use disorders (AUD) (i.e., alcohol abuse and dependence) are a complex phenotype resulting from interplay among polygenic, sociocultural, and environmental factors. The biological and behavioral heterogeneity demonstrated in AUD are only partially captured by current diagnostic classifications such as those provided by DSM-IV or ICD-10 criteria. Therefore, efforts are underway to define new classification schemes for AUD based on genetic/biological, physiological, and behavioral endophenotypes (e.g., Hines et al., 2005; Moss et al., 2007). We agree with such efforts and their premise that such heterogeneity and complexity can be best studied in more etiologically homogeneous AUD subgroups, which may be characterized by specific endophenotypes, each potentially carrying a differential type or level of genetic susceptibility for AUD. The search for phenotypic markers of risk for AUD has led to identification of several biological variables, including for example, reduced behavioral response to alcohol (Schuckit et al., 1988) and a reduced P300 component of event-related brain potential (for review, see Begleiter and Porjesz, 1999). However, despite extensive investigation in this field, health professionals do not have

reliable measures that gauge the risk of developing AUD or experiencing relapse, indicating that further research in this area is needed.

Here, we describe findings of in-vivo brain proton magnetic resonance spectroscopy (^1H MRS) studies in AUD, which, we propose, should be further explored and expanded regarding their value as a potential endophenotype for AUD. In contrast to cognition or psychosocial factors, for example, a potential MRS-derived endophenotype is more proximal to the biological mechanisms that underlie risk for the development and maintenance of substance use disorders or risk of relapse, such as disturbances in neuronal metabolism, cell membrane function (plasticity) or neurotransmission. The advantages of brain metabolite imaging are that the derived neurobiological measures are objective and, if performed carefully, are not influenced by situational or motivational factors that may affect cognition or accuracy of self-report measures. While these advantages have been attributed to functional MR imaging (fMRI) studies as well, they may be even more apparent with brain ^1H MRS measures, which are not subject to the individual variability that is inherent to functional imaging under task-based conditions. As genetic research will continue to identify polymorphisms in a great variety of different genes that may contribute to a predisposition for AUD, related neurobehavioral disorders, and relapse, in vivo MRS, as part of the emerging field of “imaging genomics,” may provide readily accessible and objective neurobiological measures that successfully link genotypes to neurocognition and psychiatric symptomatology in relatively small patient cohorts. With total brain volume as well as white and gray matter volume being highly heritable (Winterer and Goldman, 2003), it is tempting to speculate that MRS-observable metabolites, which are thought to be associated with specific cell types and molecular processes and, thus, to underlie regional morphology, are also under some degree of genetic influence.

^1H MRS is a noninvasive neuroimaging method that can assess neurochemistry by measuring concentrations of various endogenous compounds in well-circumscribed brain regions and functionally specific neural pathways in clinical and non-clinical populations. MR spectral abnormalities are easily identifiable in the presence of gross structural abnormalities such as tumors and stroke, where they contribute valuable information to the biochemical disposition of the affected brain tissue. Additionally, MR spectroscopy can identify significant and functionally relevant biochemical abnormalities that occur in the presence of subtle structural irregularities, as is often the case early in the course of many neuropsychiatric and substance use disorders. Finally, ^1H MRS can provide highly localized information regarding the functional integrity of brain tissue comprising regions/circuits implicated in the manifestation and/or maintenance of symptoms associated with the disorder in question. In the case of AUD, the variability in the severity and chronicity of the disorder as well as the presence of comorbid conditions (e.g., mood disorders, chronic cigarette smoking, and other conditions tightly coupled to the “typical” individual with AUD and not necessarily leading to research study exclusion) may lead to considerable within-group variance. Thus, regional metabolite patterns and concentration levels we measure by ^1H MRS in those afflicted with AUD are likely influenced by premorbid biological and comorbid medical, psychiatric, and substance use disorders. As many of these comorbidities are at least partially related to genetic factors, some of the brain metabolite (and neurocognitive) abnormalities we observe may indeed be pre-morbid in nature. Therefore, accounting for genetic variables can potentially increase our understanding of how genetic factors, such as single nucleotide polymorphisms (SNP) or family history of AUD (FHA), may relate to the individual variability observed in brain metabolite levels and the pattern of neurobiological and neurocognitive abnormalities found in AUD.

¹H MRS METABOLITES AND THEIR PURPORTED FUNCTIONS

Magnetic resonance spectroscopy enables the measurement of aspects of alcohol-induced brain injury that may accompany or precede alcohol-induced morphological changes. ¹H MRS allows noninvasive and concurrent quantitation of about 10 naturally occurring intracellular metabolites from throughout almost the entire brain (Ross and Bluml, 2001; Vion-Dury et al., 1994). The most intense ¹H MRS signal is usually from N-acetylaspartate (NAA), an amino acid found in high concentrations in axons and dendrites of neurons, particularly in pyramidal neurons (Birken and Oldendorf, 1989; Moffett et al., 1991; Simmons et al., 1991) and virtually absent in mature glial cells (Moffett et al., 1991). MRS-derived NAA concentration is thought to reflect neuronal viability (Vion-Dury et al., 1994) with decreased levels reflecting neuronal loss, atrophied dendrites and/or axons, reduced synaptic density, or derangements of neurometabolism (De Stefano et al., 1995; Hugg et al., 1996; Ross and Bluml, 2001; Schuff et al., 2001). NAA therefore has been ascribed significance in not only neuronal function (Dautry et al., 2000; Demougeot et al., 2001) and mitochondrial energy function (Bates et al. Neuroreport 1996) but also osmoregulation (Taylor et al., 1995). NAA increases linearly with rates of oxygen consumption and has been linked to high-energy phosphate metabolism (Pan and Takahashi, 2005) and, in the hippocampus, to glutamate (Glu) concentration in the hippocampus (Petroff et al., 2002). The ¹H MRS signal from choline-containing metabolites (Cho), which encompass choline, acetylcholine, phosphocholine, and glycerophosphocholine, represents compounds primarily involved in cell membrane breakdown and/or synthesis (Barker et al., 1994) and myelination (Ross and Bluml, 2001), reflecting cellular membrane turnover and density (Miller et al., 1996). The signal from myoinositol (mI) represents its bioactive form as a constituent of phosphatidylinositol, an important component of the phospholipid bilayer that constitutes all eukaryotic cell membranes. mI is also suggested to be an astrocyte marker and osmolyte (Brand et al., 1993; Ross and Bluml, 2001; Schweinsburg et al., 2000). The signal from creatine-containing metabolites (Cr) corresponds to the sum of concentrations of intracellular creatine and phosphocreatine, both of which are involved in the bioenergetics of neuronal and glial tissues (Ferguson et al., 2002; Ross and Bluml, 2001; Vion-Dury et al., 1994).

More recently, the advent of high-field MR magnets (>2 Tesla) has facilitated ¹H MRS measurement of gamma-aminobutyric acid (GABA) and Glu in human brain. As GABA is synthesized from Glu, their concentrations may be linked or they may be in biochemical equilibrium. Although the MR-measured amino acid levels represent the metabolically available cellular pools (which are much larger than the respective neurotransmitter pools), they have been described to be in tight chemical equilibrium with extracellular synaptic neurotransmitter levels (Rothman et al., 2003). In animals, nonspecific increases in brain GABA levels following upregulated GABA neurotransmission are directly associated with reward and nicotine self-administration (Markou et al., 2004), and extracellular Glu release in select brain regions has been associated with stronger cocaine seeking behavior in rats (McFarland et al., 2003). In humans, a recent ¹H MRS study linked low basal Glu concentrations in the striatum to decline in cognitive test performance with normal ageing (Zahr et al., 2008). Taken together, these studies relate behavior and function, which almost certainly have a genetic contribution, to regional basal concentrations of GABA and Glu as measured by in vivo ¹H MRS.

SUMMARY OF ¹H MRS FINDINGS IN AUD

A Brief Literature Review (for a More Detailed Review See, e.g., Durazzo and Meyerhoff, 2007)

Neuronal dysfunction in the frontal cortex of abstinent alcohol-dependent individuals was first suggested by lower NAA measures in frontal versus parietal cortices (Fein et al., 1994). Subsequent ¹H MRS studies in alcoholics abstinent for 3 to 40 days found lower NAA levels in the frontal lobes (Bendszus et al., 2001; Jagannathan et al., 1996), thalamus (Jagannathan et al., 1996), and cerebellum (Parks et al., 2002; Seitz et al., 1999) suggesting neuronal injury, atrophied dendrites and/or axons, and/or mitochondrial dysfunction. Others reported lower cerebellar Cho and elevated mI relative to light-drinking controls in anterior cingulate, thalamus, and frontal and parietal white matter (Barker et al., 1994; Hugg et al., 1996; Schweinsburg et al., 2000), metabolic abnormalities that were not observed in 6-year-abstinent alcoholics (Schweinsburg et al., 2000, 2001). Thus, they suggest recovery of membrane abnormalities or osmotic changes with long-term abstinence from alcohol. Lower concentrations of NAA in frontal white matter and of NAA, Cho, and mI in the cerebellum correlated with lower neurocognitive and motor functioning in short-term abstinent alcoholics (e.g., Bendszus et al., 2001; Parks et al., 2002). Recent research in community-dwelling hazardous drinkers also found lower NAA levels in frontal white matter, suggestive of axonal injury (Meyerhoff et al., 2004). These lower NAA concentrations were associated with poorer performances on measures of executive skills and working memory as well as lower frontal P300b amplitudes.

In a small sample of recently detoxified alcoholics, occipital cortex GABA levels measured by ¹H MRS were 30% lower than in nonalcoholic controls, consistent with plasma and cerebrospinal fluid GABA levels (Adinoff et al., 1995). More detailed studies showed that GABA levels were elevated in 1-week abstinent alcoholics and reduced at 4 weeks (Mason et al., 2006). Elevated Glu concentrations have been associated with neurotoxicity, and in animal models of alcohol withdrawal, Glu levels are increased (for review, see Bleich et al., 2004). However, as far as we know, there are no reports of brain ¹H MRS Glu measurements in AUD.

The noninvasive nature of ¹H MRS studies allows repeated scanning to assess the time course of regional metabolite concentration changes during sobriety. Such longitudinal studies focused primarily on frontal lobes and cerebellum. Metabolite signal increases in these regions over 3 to 9 weeks of abstinence suggested remyelination (Bartsch et al., 2007; Bendszus et al., 2001; Ende et al., 2005; Martin et al., 1995) and increases of frontal NAA measures suggested recovery from neuronal injury or dysfunction (Bartsch et al., 2007; Bendszus et al., 2001).

Brain MRS-derived metabolite measures in AUD often correlate with neurocognitive measures, reinforcing their functional significance. For example, at 1 month of abstinence, higher frontal NAA/Cr was related to better auditory-verbal memory, while higher cerebellar NAA/Cr positively correlated with attention/concentration (Bendszus et al., 2001). NAA concentration increases in the cerebellar vermis over 3 months of sobriety were related to improving auditory-verbal learning (Parks et al., 2002), while increasing mesial frontal NAA related positively to improving attention (Bartsch et al., 2007).

Our MRS Research in Treatment-Seeking AUD (for a More Detailed Account See Durazzo et al., 2007b)

As chronic smoking is highly comorbid in alcohol dependent populations (70 to 90% depending on study and cohort investigated), we have recently focused on the effects of comorbid chronic smoking on brain biology and function in alcoholics via MR and

neurocognitive assessments. We measured regional concentrations of NAA and Cho using ^1H MRS imaging in 1-week-abstinent, smoking and nonsmoking treatment-seeking alcoholics (Durazzo et al., 2004). We observed that compared with smoking and nonsmoking light-drinking controls, alcohol dependence was associated with lower frontal NAA and Cho levels, as well as lower parietal and thalamic Cho. Chronic cigarette smoking was associated with lower midbrain NAA and Cho and with lower cerebellar vermis Cho. More specifically, in 1-week-abstinent alcoholics, smoking was associated with lower NAA concentrations in the frontal white matter and midbrain as well as lower midbrain Cho. In addition, smoking alcoholics showed trends to lower NAA in the parietal gray matter and lenticular nuclei relative to nonsmoking alcoholics. These differences of regional metabolite concentrations remained significant after controlling for the greater drinking severity in smoking alcoholics. Numerically, smoking alcoholics evidenced the lowest NAA and Cho levels of all 4 groups in virtually all brain regions evaluated by multislice ^1H MRS imaging.

In longitudinal ^1H MRS imaging investigations, we assessed regional metabolite changes in treatment-seeking alcoholics over the first month of sobriety following detoxification (Durazzo et al., 2006; Gazdzinski et al., 2008b). Results indicated that the nonsmoking alcoholics showed increased NAA in the frontal white matter and Cho in neocortical gray matter in all but the occipital lobes; nonsmokers also demonstrated significant Cho increases in the white matter of all lobes. Furthermore, in nonsmoking alcoholics, NAA and Cho levels in medial temporal lobe, which includes the hippocampal formation, normalized to control levels over 1 month of abstinence. By contrast, smoking alcoholics over 1 month of abstinence from alcohol exhibited increasing NAA and Cho only in the frontal lobe, while NAA decreased in the parietal and occipital white matter and did not change in medial temporal lobe. Taken together, our studies in treatment-seeking alcoholics showed significant influences of chronic smoking on AUD-related brain injury and recovery thereof. Cognitive studies performed in the same population demonstrated the functional relevance (clinical significance) of these smoking effects on neurobiology (Durazzo et al., 2006, 2007d). Consistent with these cognitive effects of chronic smoking in recovering alcoholics, recent analyses also showed neurocognitive deficits in nontreatment seeking hazardous drinkers with (Durazzo et al., 2007c) and without (Durazzo et al., 2007a) human immunodeficiency virus infection.

In regard to the functional significance of our MRS-derived metabolite concentrations, we observed that the relationships between brain metabolites and neurocognition are different in pattern and/or magnitude between smoking and nonsmoking alcoholics (Durazzo et al., 2004). These differences may give clues to the impact of comorbid conditions, such as smoking, on functional neurocircuitry. In 1-week-abstinent, nonsmoking alcoholics, cerebellar vermis NAA was positively related to visuospatial learning and visuospatial memory, whereas in smoking alcoholics, cerebellar vermis NAA was positively related to visuomotor scanning speed and incidental learning. Over 1 month of abstinence from alcohol (Durazzo et al., 2006) in nonsmoking alcoholics, improvements in visuospatial learning and memory, working memory, and visuomotor scanning speed and incidental learning were related to increases of NAA and Cho concentrations in the frontal and parietal lobes and in the thalamus. For smoking alcoholics, however, only NAA increases in midbrain and caudate correlated with improving visuospatial learning and memory. Within the medial temporal lobe of both smoking and non-smoking alcoholics, increasing Cho, Cr, and mI levels correlated with improving visuospatial memory (Gazdzinski et al., 2008b). Overall, these significant metabolite-function relationships underscore the functional significance of ^1H MRS measures and their modulation by smoking status. Future research may assess the role of genotypes on these relationships.

Being noninvasive, ^1H MRS is ideal for therapeutic monitoring of disease progression, regression, and neurotherapeutic decision making (Lin et al., 2005). As described above, in vivo ^1H MRS measures assess neurobiological change during abstinence from AUD, and can be obtained from neural circuits that support various aspects of cognition (e.g., dorsolateral cortical-subcortical and fronto-thalamo-ponto-cerebellar circuitry; Cummings, 1998; Sullivan, 2003) and the development and maintenance of substance use disorders (Kalivas and Volkow, 2005). The degree of neurobiological abnormalities following acute detoxification from alcohol and the variable levels of recovery during abstinence may not only be influenced by prevalent comorbid conditions (e.g., smoking, mood disorders, hypertension) but also they may be partially influenced by genotype as well. This prompts the question if variable treatment response reflects different MRS-phenotypes with various degrees of neurologic resilience and/or plasticity. The new field of MRS-genetics is positioned to provide new insights into these important relationships.

AUD, A COMPLEX BUT TREATABLE DISORDER

Probing for genetic influences on neurobiological changes in AUD cannot ignore the potential influence of medical, psychiatric and substance misuse comorbidities. The most frequently used substances among individuals with AUD are tobacco products (reviewed in Meyerhoff et al., 2006), and approximately 80% of alcohol-dependent individuals in North America are regular smokers (Hurt et al., 1994; Pomerleau et al., 1997; Romberger and Grant, 2004). Several theories attempt to explain the concurrent heavy use of alcohol and tobacco products: potentiation of each other's rewarding properties (Barrett et al., 2006; Le et al., 2003; Narahashi et al., 2001; Rose et al., 2003), counteracting the adverse effects of acute alcohol on cognition and motor incoordination (Prendergast et al., 2002), or existence of classical conditioned cue reactivity, leading to simultaneous craving for both substances (Drobes, 2002). A genetic susceptibility for concurrent cigarette smoking and AUD has also been proposed (Le et al., 2006; Madden and Heath, 2002; Wilhelmsen et al., 2005). Further genetic studies in AUD will enhance our understanding of how smoking and alcohol interact to influence central nervous system activity (e.g., Bierut et al., 2004; Le et al., 2006; Madden and Heath, 2002; Miksys et al., 2003; Tyndale, 2003) and of the development and maintenance of substance dependence. Dopamine (DA) neurotransmission and the mesocorticolimbic system have critical roles in this interaction (Baler and Volkow, 2006). Although alcohol and nicotine exert their adverse effect on brain neurobiology through different mechanisms, the initiation and maintenance of dependence for substances of abuse involves the "reward circuit" of the mesocorticolimbic system (Baler and Volkow, 2006). Thus, genes or polymorphisms of genes that are related to DA and potentially serotonergic and glutamatergic transmission (Flatscher-Bader et al., 2008) are promising targets of brain MRS-genetics studies in AUD.

AUD is also characterized by a very high prevalence of psychiatric and substance abuse comorbidities (Fein et al., 2007; Hasin et al., 2007; Hines et al., 2005; Kessler et al., 1997; Kranzler and Rosenthal, 2003). Alcohol dependence, bipolar disorder, posttraumatic stress disorder, and cluster B personality disorders may share common genetic determinants of novelty-seeking, impulsivity and compulsivity, harm avoidance, and reward dependence. Increasing evidence suggests that such premorbid personality traits may predispose individuals to addictive behavior (e.g., Dalley et al., 2007 and references cited therein). Along those lines, certain genetic variants have been associated with both AUD and psychiatric disorders, such as anxiety (Enoch et al., 2003). A DA receptor D4 polymorphism, for example, has been linked to attention-deficit/hyperactivity disorder, schizophrenia, and alcohol craving (Hutchison et al., 2002), whereas a single polymorphism of the serotonin transporter (5HTT) gene has been linked to alcohol dependence, major depressive disorder, anxiety, and suicide attempts (Hines et al., 2005).

CANDIDATE GENES IN AUD POTENTIALLY RELATED TO BRAIN ^1H MRS METABOLITES

Principal Considerations

It has been a dream to relate specific cognitive test performance to different genotypes, but measuring cognition precisely and objectively for statistically valid group comparisons requires relatively large samples. This is rationalized because cognition/behavior is very distant from genotypes on an imaginary scale that connects gene expression to clinical features (see e.g., Roffman et al., 2006). It has been shown that fMRI responses to specific tasks, however, appear to be more sensitive than cognition/behavior itself to different genotypes, sometimes requiring less than 20 probands to establish a gene-fMRI response relationship. Proton MRS-derived regional metabolite concentrations are related to cognition in a variety of diseases and in relatively small samples (see above). Therefore, the naturally occurring biological range of regional MRS measures may increase the effect size of genotypic influences on neurobiology related to cognition, behavior, and clinical features in general. In other words, there are likely to be stronger gene effects on observable differences of brain metabolite concentrations than on cognition, behavior, or clinical features, because the latter are so much further downstream from genetic variation than neurobiology. Similarly, regional metabolite measures are closer to neurochemical pathways than regional brain volumes on our imaginary scale connecting genetic variation to clinical features. Thus, they should be more sensitive to genotypic influences than brain structural measures.

Functional MRI-genetics studies have been conducted for several years now to bridge the association between gene expression and behavior (see e.g., Hariri and Weinberger, 2003; Roffman et al., 2006). Other neuroimaging-genetics studies involving MRS or MRI volumetrics have been rare. We refer the interested reader to the fMRI-genetics literature to learn about the rationale and principles underlying these studies, critical general factors for selection of appropriate candidate genes from the human gene pool, and about well-formulated ideas on the general role of neuroimaging vis-à-vis intermediate phenotypes. It has been widely recognized that our genes influence cognitive function and behavior, which are related to specific regional metabolite concentrations in healthy controls, AUD, and other pathologies. Thus, one could argue that genes may also affect basal metabolite levels, their regional specificity within the brain, and/or perhaps spatial metabolite patterns in addiction-related neural pathways that can be measured by ^1H MRS. Studying these relationships will help our understanding of how genes affect the development and maintenance of AUD, their brain altering characteristics (e.g., neurotransmission), and the restoration of normal brain biology and function with long-term abstinence from alcohol and/or co-abused substances (i.e., cerebral plasticity).

Collecting specific genetic information will assist in determining if research participants with AUD and healthy controls with particular SNP demonstrate neurobiologic and neurocognitive characteristics different from those who do not have these genetic predispositions. Here, we discuss just a few candidate genes, in no particular order, that possibly confer genetic susceptibility for AUD and related neurobehavioral disorders and, at the same time, may be directly linked to the concentrations and/or regional patterns of MRS-detectable metabolites. While we focus here on “the usual suspects”, other functional polymorphisms linked to AUD and its comorbid disorders may also influence MRS-derived metabolite levels. Recent analyses reveal common pathways and genes related to addiction on a variety of substances (alcohol, cocaine, opioids, and nicotine) (Li et al., 2008; Uhl et al., 2008), so that the following descriptions may also relate to drugs of abuse other than alcohol.

Apolipoprotein E

Apolipoprotein E (APOE) is implicated in neuronal repair and remodeling. The $\epsilon 4$ allele is related to abnormalities in the cytoskeletal system (e.g., microtubules and microfilaments), the ability of neurons to maintain and re/establish cellular connections, and cerebrovascular characteristics, and it has been associated with increased risk for various neurodegenerative diseases, including Alzheimer's disease. Individuals who are homozygous for, or carriers of the $\epsilon 4$ allele have a greater risk of developing abnormal cognitive decline and depressive symptomatology with advancing age (Bretsky et al., 2003; Caselli et al., 2004) and of developing mild cognitive impairment in old age after higher alcohol consumption in middle age (Jorm et al., 2007 and references cited therein). $\epsilon 4$ carriers with hazardous alcohol consumption also perform worse on some cognitive tasks than $\epsilon 4$ carriers who drink less, whereas noncarriers drinking at hazardous levels perform better than non/light drinkers (Jorm et al., 2007). As biological effects of AUD on the brain may mimic those of accelerated aging, the presence of APOE $\epsilon 4$ may constitute a risk factor for alcohol-related brain injury as expressed specifically in abnormal regional concentrations of NAA reflecting neuron and Cho reflecting membrane viability. The $\epsilon 4$ allele has been related to hippocampal atrophy in female alcoholics (Bleich et al., 2003), but only in the presence of high plasma homocysteine levels (Wilhelm et al., 2007). Possibly underlying these morphological abnormalities in the medial temporal lobe of alcoholics are neurometabolic changes detectable by ^1H MRS (Gallinat et al., 2007; Gazdzinski et al., 2008) (but see Bartres-Faz et al., 2002).

Brain-Derived Neurotrophic Factor and Other Development-Related Genes

Brain-derived neurotrophic factor (BDNF) supports the survival and growth of many neuronal subtypes and has emerged as a critical factor mediating synaptic efficiency and behavior-related plasticity (Lipsky and Marini, 2007), neuronal connectivity (Cotman and Berchtold, 2002), as well as changes in brain volume and hippocampal structures (e.g., Ho et al., 2007). As such, it is thought to influence learning and memory (Egan et al., 2003; Frodl et al., 2007; Ho et al., 2006; Oroszi et al., 2006), which have been shown to be related to MRS-derived metabolite concentrations. In particular, one SNP of BDNF (Val66Met and a valine to methionine substitution) may influence MRS-derived measures of the neuronal marker NAA in AUD and controls. One of the first peer-reviewed studies that analyzed MRS outcome measures as a function of genotype revealed that in healthy humans, individuals expressing the methionine allele show poorer episodic memory (i.e., medial temporal cortex based declarative memory), lower NAA in the hippocampus, and abnormal hippocampal activation (Egan et al., 2003). Egan's study can be considered prototypic for MRS-gene studies advocated here and it suggests a pathway by which functional variations of genes can be tied to various complex phenotypes, including MRS and cognition.

As the BDNF methionine allele appears associated with injury to hippocampal neurons, recovery of neuronal function during abstinence from alcohol may also be mediated by BDNF genotype. Thus, treatment that is aimed at reconstituting neuronal integrity, e.g., via cognitive enhancers, may be differentially successful in patients with different BDNF genotypes. The ability to predict treatment response based on genotype and MRS biomarkers promises to be of great clinical value.

In addition to BDNF, many other neurotrophic factors and associated genes critical for neuronal survival, maintenance, and development may be worthwhile considering in relation to MRS-derived measures of neuronal viability and support. For example, Semaphorin (SEMA4F) has been shown to play a crucial role in axonal growth cone guidance (Goldberg and Weinberger, 2004) and the human activity-regulated cytoskeleton-associated gene regulates synaptic transmission/plasticity (Kremerskothen and Barnekow, 2000). Another

candidate gene that plays an important role in hippocampal development of healthy individuals is disrupted-in-schizophrenia-1 (DISC1), expressed primarily in the hippocampus. It has been related to performance on the Wisconsin Card Sorting Test, logical memory, hippocampal structure, and left hippocampal NAA and allelic variations within DISC1 increase the risk for schizophrenia (Callicott et al., 2005). The hippocampal formation is a critical brain region in the etiology of AUD, making DISC1 a promising candidate gene for MRS-gene studies.

Dopamine Receptor Genes and μ -Opioid Receptors

The neural substrate underlying addictive behavior and reward is the mesolimbic DA system. In cocaine abusers and in alcohol dependent individuals, DA D2 receptor availability is reduced in the basal ganglia (Volkow et al., 1993, 2006), either as a predisposing trait or as a consequence of chronic substance use. A recent study links the impulsivity trait in drug-naïve rats to lower DA D2/3 receptor availability in the nucleus accumbens (Dalley et al., 2007) and suggests that DA genes may influence impulsive brain disorders such as drug, alcohol, or nicotine addiction. We propose that genetically influenced dysfunctional DA neurotransmission may be associated with abnormal levels and regional patterns of various MRS-observable metabolites (including those of the neuronal marker NAA and Glu) in brain regions encompassing the mesolimbic and mesocortical DA pathways. A case in point is a recent study that relates DA transporter genotype to higher relative NAA measures (i.e., neuronal viability) in the left putamen of both healthy controls and patients with obsessive compulsive or bipolar disorder (Scherk et al., 2007).

The activity of the mesolimbic DA system, including DA release, is thought to be modulated by endogenous opioids. A functional SNP of the gene for the μ -opioid receptor (OPRM1) appears to have a significant impact on cue-induced craving for alcohol in heavy drinkers (van den Wildenberg et al., 2007), response to naltrexone treatment in alcohol dependent patients (McGeary et al., 2006; Oslin et al., 2003), as well as responses to alcohol (Barr et al., 2007; Ray and Hutchison, 2004). Thus, specific OPRM1 genotypes may be associated with differential DAergic activity in the mesolimbic areas of the brain that in turn may be related to neuronal viability detectable by ^1H MRS. Other genes that affect DAergic activity and degeneration of DAergic neurons, such as monoamine oxidases, may also be linked to neuronal viability.

Catechol-O-Methyltransferase

Catechol-O-methyltransferase (COMT) is an enzyme that metabolizes catecholamines such as norepinephrine and DA. An important functional SNP results from the substitution of methionine (Met) for valine (Val) at codon 158 (Val158Met) (Lachman et al., 1996). COMT is thought to be involved in DA degradation with high (Val) and low enzyme activity (Met), thus affecting synaptic DA levels. As a high level of DA transmission occurs in frontal cortex, its modulation by the functional COMT polymorphism may influence executive function and neurophysiology based on/related to abnormalities in anterior frontal-subcortical circuitry (Mega and Cummings, 1994; Saint-Cyr, 2003; Starr et al., 2007). The COMT Val158Met genotype has been associated with poorer executive function in healthy controls and in schizophrenia (Egan et al., 2001), as well as in traumatic brain injury (Lipsky et al., 2005). The same functional polymorphism has been correlated with AUD in Caucasians. And because AUD is characterized by lower prefrontal cortical DA and relative poor executive performance, COMT genotype may be related to frontal cortical NAA, Glu, and/or GABA concentrations in AUD and perhaps to their distributions within the frontal lobe.

Research in healthy controls generally found no effects of COMT Val158Met on volumes of total gray or white matter (Zinkstok et al., 2006) or on frontal lobe volume (Ho et al., 2005). However, healthy Val158 allele homozygotes had smaller hippocampi and temporal lobe volumes (Taylor et al., 2007) and Val allele homozygotes with schizophrenia also showed smaller temporal lobe structures (Ohnishi et al., 2006). To the best of our knowledge, no published studies so far have related COMT genotype to brain metabolite concentrations. However, because the latter often show strong and numerically large correlations with neurocognition (see above), we predict modulation of regional metabolite concentrations by COMT genotype.

Glutamate Receptor Genes

Common polymorphisms of Glu receptors may play a role in Glu-mediated excitotoxicity observed in human addiction, anxiety disorders, associated learning, and cognitive behavior. Through its ability to measure Glu, glutamine, and the neuronal marker NAA in human brain tissue as well as their changes with disease and treatment, MRS can investigate potential links between Glu neurobiology/excitotoxicity and specific Glu receptor genotypes. Other genes reflecting Glu dysfunction, such as metabotropic Glu receptor mgluR3 (GRM3) and G72, which are thought to play a role in schizophrenia, may also be linked to AUD and to treatment aimed at affecting glutamatergic neurotransmission (Roffman et al., 2006). In healthy volunteers, the A/A genotype of GRM3 was associated with a lower NAA-over-Cr ratio in the right dorsolateral prefrontal cortex compared to the G carriers, suggesting genetically regulated Glu neurotransmission and mitochondrial activity (Egan et al., 2004; Marenco et al., 2006).

5HTT Genes

Brain morphometry and function as well as risk of disease are altered by functional 5HTT polymorphisms (Frodl et al., 2004; Hu et al., 2006; McEwen and Magarinos, 1997 and references cited therein). Serotonin dysregulation (e.g., by stress) diminishes neurotrophic factors critical to hippocampal neurogenesis and increases hippocampal glutamate activity to excitotoxic levels. The S allele of the 5HTT gene is associated with poorer memory function and higher waking cortisol in older adults (O'Hara et al., 2007) and with lower hippocampal NAA concentration in healthy controls (Gallinat et al., 2005). The L/L genotype is associated with smaller hippocampal volumes in depressed patients compared with nondepressed controls with the same genotype (Frodl et al., 2004). Furthermore, 5HTT polymorphisms have been related to depression severity, duration, and poorer response to selective serotonin reuptake inhibitor therapy than to other medication as well as to anxiety-related behavior (e.g., Enoch and Goldman, 2001). As a large fraction of individuals with AUD also display depressive and/or anxiety symptomatology and possibly higher stress levels, hippocampal structural and spectral changes in AUD may be influenced by 5HTT genotype. A particular L/L genotype has also been shown to be associated with increased risk for obsessive compulsive disorder (OCD) (Hu et al., 2006). AUD has been described to have characteristics of compulsive behaviors and, just as OCD (Sumitani et al., 2007 and references cited therein), has been associated with lower NAA concentrations in the anterior cingulate. This 5HTT promoter polymorphism has also been linked to consumption of both alcohol and tobacco in adolescents (Skowronek et al., 2006). Taken together, genetic variants of the 5HTT genes may also link to prefrontal neurobiology in AUD and related frontal lobe-mediated executive function.

EXPERIMENTAL DATA SUPPORTING GENETIC EFFECTS ON MRS MEASURES

Effects of FHA on Regional Brain Metabolites

To assess if MRS-derived metabolite measures are at least in part modulated by genetic factors, we analyzed our metabolite data from different alcohol-consuming populations (nontreatment-seeking hazardous drinkers recruited from the community, treated alcoholics in recovery, and non/light-drinking controls) as a function of FHA. A FHA effect on brain metabolite data could reflect premorbid (i.e., genetic and/or environmental) factors rather than sequelae of years of heavy drinking. To assist in distinguishing between genetic or environmental etiologies, it will be necessary to identify genotypes that predict both AUD and brain MR measures. And to determine if MR measures are stronger predictors of AUD risk than FHA itself, we need to determine if nonalcoholic children of alcoholics have these abnormal MR measures. If indeed so, we are a step closer to a new MR endophenotype.

Studies that evaluated effects of FHA on brain biology and function show that family-history-positive (FHP) individuals are at higher risk for early development of AUD than their family-history-negative (FHN) counterparts (Cloninger, 1987; Schuckit, 1984; Tarter et al., 1985). Reports in children and adolescents suggest that a positive FHA contributes to impaired cognition and lower P300 event-related potential amplitude (Berman et al., 1993; Drejer et al., 1985; Hill and Steinhauer, 1993; Polich et al., 1994). Furthermore, nonalcoholic FHP adults also have a different sensitivity to acute alcohol than low-risk individuals (e.g., Newlin and Thomson, 1999). Furthermore, although alcoholics have low striatal DA D₂ receptor availability, FHP *nonalcoholic* individuals have *higher* levels of striatal dopamine D₂ receptors than their FHN counterparts, (which are associated with glucose metabolism, a measure of brain function, in anterior cingulate, prefrontal and orbitofrontal cortices and) which may protect against the development of AUD (Volkow et al., 2006). (To the best of our knowledge, the influence of FHA on DA D₂ receptor availability in alcoholics is unknown.) These types of studies that classify research participants according to their FHA imply that unidentified genes contribute to their observations. In vivo ¹H MRS, with its ability to directly measure aspects of brain biology that may be altered by FHA, can further help illuminate genetic influences on brain biology and function.

We studied by ¹H MRS nontreatment-seeking hazardous drinkers recruited from the community (heavy drinkers, HD) and compared them to light drinkers (LD, who drank on average 13 drinks per month over lifetime) (Meyerhoff et al., 2004). Individuals with at least 1 parent identified by the research participant as a problem drinker were defined as FHP. Of the 46 HD participants, 18 were FHN and 28 FHP. Drinking severity (i.e., monthly drinks over the past year, the last 3 years, and lifetime) was similar between FHP and FHN HD, with approximately 200 drinks per month. After covarying for age, we found that frontal white matter NAA in FHN HD was 6% lower than in FHP HD and 8% lower than in FHN LD (both $p < 0.05$) and frontal gray matter NAA tended to be lower in FHN compared with FHP HD ($p = 0.07$). Similarly, frontal gray matter Cr and mI, as well as parietal white matter Cr, were at least 10% lower in FHN versus FHP HD (all $p < 0.07$). No significant lobar NAA, Cr, and mI differences were observed between FHP and FHN LD groups. However, brainstem mI was 15% higher in 17 FHN LD versus 19 FHP LD ($p = 0.01$) and 22% higher in 18 FHN HD versus 28 FHP HD ($p = 0.01$). Thus, across all participants independent of drinking status, FHN individuals had 18% higher brainstem mI than FHP participants ($p = 0.0004$). These results are confounded by a different sex distribution within groups and need to be considered preliminary. Nevertheless, they raise the possibility that a positive FHA imparts some degree of protection against alcohol induced frontal neuronal injury (as

indicated by NAA measures) and brainstem gliosis (as indicated by mI measures) in alcoholic HD or, conversely, that FHN HD are more vulnerable to MRS-detectable brain injury than FHP HD. Protective effects of a FHA on brain neurobiology is consistent with our volumetric MRI findings of significantly smaller CSF volumes (as a percent of intracranial volume) in frontal, parietal, and occipital lobes of a similar sample of FHP versus FHN HD, suggesting less brain tissue loss in FHP versus FHN HD (Cardenas et al., 2005). Only further prospective studies will show how these early results can be consolidated with other findings of the effects of FHA on brain biology and function described above.

In our treatment-seeking alcohol-dependent cohort (ALC), composed primarily of male U.S. Armed Services Veterans, over 90% of participants identified at least 1 parent as a problem drinker. Therefore, group comparisons between FHP and FHN participants require larger samples than currently available from our neuroimaging studies. However, the family density of AUD, which is the prevalence of problem-drinking among first- and second-degree relatives of the alcoholic participant (Stoltenberg et al., 1998), does show considerable variability across our ALC participants. Family density of AUD is an extension of FHA, where weights are assigned to familial problem-drinkers, according to their genetic relatedness to the ALC participant (Stoltenberg et al., 1998; Zucker et al., 1994). In 55 1-month abstinent ALC, we observed that greater family density of AUD was positively related to higher NAA concentrations in total lobar white matter and specifically in the temporal lobes (both $r > 0.32$, $p < 0.05$). Greater family density also tended to be associated with better performance on measures of fine motor skills, executive skills, and processing speed (all $r > 0.26$, $p < 0.06$).

Together, our analyses in 2 different alcohol dependent cohorts suggest that alcoholics without a family history or with low density of AUD are vulnerable to neuronal injury. In other words, alcoholics with a family history or higher density of AUD may be less susceptible to alcohol-induced neuronal injury and potentially to alcohol-induced dysfunction in some areas of cognition. While these findings were unexpected, a similarly protective effect of higher striatal DA D2 receptor availability has been postulated on the development of alcoholism in nonalcoholic children of alcoholics (Volkow et al., 2006). Although FHA is not an indicator of specific genetic influences on alcohol dependence, it signals the heritability of AUD. The early analyses described here suggest that neurometabolic measures, including those that can be obtained routinely by ^1H MRS, are influenced by heritability. Therefore, prospective studies of genes, behavior, and neurometabolic patterns, a powerful triad if used properly in experimental design, are likely to advance significantly our understanding of factors underlying the development and maintenance of AUD.

Such studies may also enhance approaches to alcoholism treatment matching by identifying those individuals early in treatment who are most likely to relapse. It is well described that premorbid and comorbid factors influence behavioral and pharmacological treatment for AUD and have implications for relapse (Bottlender and Soyka, 2005; Hughes, 2002). In our AUD cohort, we tracked the alcohol consumption of 65 participants for 3 to 12 months following outpatient treatment (Durazzo TC, Gazdzinski S, Yeh P-H, Meyerhoff DJ, 2008, ICANA conference presentation, unpublished data). At 1 month of abstinence, we observed that those participants who ultimately relapsed had lower NAA in the frontal and parietal lobes and lower Cho in the frontal GM than those who abstained. Logistical regression indicated that decreased frontal white matter NAA and frontal gray matter Cho were associated with a 3.4- and 4.8-fold increase, respectively, in the odds for relapse. Together with unipolar mood disorders and processing speed, the regression model accurately predicted greater than 90% of relapsers and 80% of abstainers. Adding genetic factors to

similar analyses, such as COMT or OPRM1 polymorphisms, may further increase the accuracy of relapse prediction and treatment efficiency.

CONCLUSIONS

The field of MRS-genetics is in its very infancy and originated in reports of an APOC1 polymorphism predicting NAA/Cr in basal ganglia of individuals with age-related memory impairment (Bartres-Faz et al., 2002). Very little work on how genetics influence MRS-derived measures of metabolites, metabolism, and related cognitive behavior has been published in peer-reviewed journals since. This may reflect more the complex nature of a combined MRS-genetics study or the misperception that prohibitively large cohorts are needed to establish MRS-gene connections, than the lack of interest in this topic. MRS-genetics studies may serve as more sensitive predictors of risk for AUD and AUD severity than current routine clinical tools, including neuropsychological tests and rating scales. To explore the value of MRS as a valid endophenotype of AUD, neuroimagers are well advised to analyze MRS and cognitive/behavioral data of individuals with and without AUD as a function of particular genetic polymorphisms. While currently very few functional polymorphisms have been related directly to any of the MRS-observable metabolites, there is abundant evidence that genetic polymorphisms influence cognitive function. As years of MRS research have shown, various domains of cognition show clear and strong correlations with regional metabolite concentrations in many neurodegenerative diseases including AUD and in metabolic recovery from alcohol-induced brain injury during sobriety. Neuroimagers can promote progress in MRS-genetics research in AUD by banking DNA for future targeted analyses of genes that may emerge from contemporary association and linkage studies. Just as the marriage of fMRI and genotyping has revolutionized the research linking genotype to cognitive/behavioral phenotypes, specific genotypes may be linked directly to in vivo MRS-derived biological measures of localized metabolite concentrations and their spatial distributions across important neural pathways that influence cognition and behavior in AUD. An added benefit of MRS measures is their lower susceptibility to transient factors that may modulate performance on neuropsychological or cognitive measures commonly used in fMRI studies. With respect to our own work, continued assessment of genotypic markers that distinguish smokers from nonsmokers has particular applicability to our MR outcome measures and how they relate to cognition and treatment success in AUD. Identification of genotypic markers that accompany psychiatric AUD comorbidities will also have relevance for MR spectroscopic and neuropsychological assessment of AUD and recovery with abstinence from alcohol. As AUD is a complex disorder with genetic-biological-psychosocial determinants, it is the perfect testing ground for the utility of the newly emerging field of MRS-genetics. This research will not only offer clues to the functional significance and downstream effects of genetic differences in AUD, but it will also allow monitoring the efficacy of pharmacological and behavioral interventions and assist in the prediction of relapse as a function of genotype, thereby having the potential to facilitate clinical management in AUD. Before MRS can be defined as a new endophenotype, however, research will first have to demonstrate that MRS outcome measures satisfy the basic criteria of heritability and state independence.

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