PROOF

Center Line

Bowles Center for Alcohol Studies
School of Medicine, University of North Carolina at Chapel Hill

Our mission is to conduct, coordinate, and promote basic and clinical research on the causes, prevention, and treatment of alcoholism and alcoholic disease.

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Wilhelmsen Lab Elucidates the Genes that Underlie Hereditary Aspects of Alcoholism

Alcoholism has been shown to have a strong genetic influence through family, twin, and adoption studies. Alcohol use is a complex behavioral trait with contributions from an unknown number of genes that interact with environmental factors. The cumulative effects of these genes and environmental factors contribute to the characteristic behaviors that constitute the medical definition of abuse and dependency. One goal of alcohol researchers is to identify the genes that contribute to alcoholism. This may lead to reliable markers of susceptibility, and ultimately targets for drug therapies.

Wilhelmsen Research Team: Kirk Wilhelmsen, Ph.D., Scott Chasse, Ph.D., Amy Webb

Dr. Kirk Wilhelmsen, Bowles Center researcher and Associate Professor of Genetics and Neurology is engaged in the search for the genes that contribute to alcoholism. The Wilhelmsen laboratory is engaged in the genetic mapping of susceptibility loci for complex neurological diseases using automated gene mapping technologies to facilitate these efforts. As data accumulates, it will enable analysis of numerous traits for the entire genome and allow exploration of relationships between genes and behavior. The Wilhelmsen lab is applying these techniques to the genetics of alcoholism and substance abuse as well as cognitive function.

Alcohol dependence is influenced by a combination of several genes. However, each gene may only make a small contribution to the disease, making it very difficult to detect. Investigations intending to find the genes responsible for the global diagnosis of alcoholism will most likely fail without a good description of each gene's effect on behavior. Wilhelmsen explains, "Genetic epidemiology has shown a heritable component to alcoholism, but it is not clear how many genes are involved, how these genes interact with each other, or how they are affected by the environment. The pattern and quantity of alcohol consumption are easily definable traits that also have a heritable component. Mapping the genes for these simpler traits may be easier and could lead to the development of a more etiologically-based framework for the definition of alcoholism."

Linkage analysis, one of the ways scientists search for genetic markers of disease, involves sequencing portions of chromosomes. Chromosomes are tightly coiled microscopic rod-like structures of DNA and protein in cell nuclei that contain 50-245 million DNA base pairs among 22 double chromosomes and 2 sex chromosomes, eg. XX or XY. Humans have an estimated 30,000 genes within these chromosomes making linkage of portions of chromosomes with specific pathologies, including alcoholism, a very complicated task. In these studies, inheritance patterns of traits are used to localize corresponding genes. Genetic signposts, called markers, are used to tell which chromosomes were inherited from each parent. A trait is "mapped" when marker data predicts which parent passed down the trait. This process of "identity by descent" tends to provide a very coarse estimate of chromosome location because it does not specify which gene is responsible for the trait.

On the other hand, association analysis or "identity by state" is a population-based approach that compares genotypes between "affected" and "control" populations. The advantage to this approach is the ability to discover correlations between specific differences in genes and disease traits, even if the gene's contribution is relatively small. In this case the "affected" population is defined by a

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Kirk Wilhelmsen, M.D., Ph.D.

Affiliations

Associate Professor of Genetics and Neurology , University of North Carolina at Chapel Hill; Bowles Center for Alcohol Studies; Carolina Center for Genome Sciences

Education and Training

M.D., University of Wisconsin, Madison, 1986; Ph.D., Molecular Biology, University of Wisconsin, Madison, 1984; B.S., Chemistry, University of California, San Diego, 1978; Medical Intern, Neurology Resident and Postdoctoral Fellow in Human Genetics at Columbia Presbyterian Medical Center, 1986-1990.

Recent Publications

Wilhelmsen KC, Swan GE, Cheng LS, Lessov-Schlaggar CN, Amos CI, Feiler HS, Hudmon KS, Ring HZ, Andrews JA, Tildesley E, Benowitz NL, Hops H. Support for previously identified alcoholism susceptibility loci in a cohort selected for smoking behavior. Alcohol Clin Exp Res. 2005 Dec;29(12):2108-15.

Schuckit MA, **Wilhelmsen KC**, Smith TL, Feiler HS, Lind P, Lange LA, Kalmijn J. Autosomal linkage analysis for the level of response to alcohol. Alcohol Clin Exp Res. 2005 Nov;29(11):1976-82.

Ehlers CL, Gilder DA, Wall TL, Phillips E, Feiler H, **Wilhelmsen KC**. Genomic screen for loci associated with alcohol dependence in Mission Indians. Am J Med Genet B Neuropsychiatr Genet. 2004 Aug 15;129(1):110-5.

Ehlers CL, **Wilhelmsen KC**. Genomic scan for alcohol craving in Mission Indians. Psychiatr Genet. 2005 Mar;15(1):71-5.

Website

http://genomics.unc.edu/wilhelmsen/wilhelmsen_dw.htm

specific trait such as alcohol craving, withdrawal, or binge drinking. It has been impractical to systematically search all genes for association. In most cases association analysis has been limited to "candidate" genes.

The identity by descent, or linkage methods, use different genetic information than identity by state, or association methods. This allows the two methods to be used sequentially in subjects. Initially, Dr. Wilhelmsen uses traditional disease definitions of alcoholism to search for disease loci or specific chromosomal locations. (Figure) These locations are linked to susceptibility as measured by documented linkage scores. The trait definitions are refined in an attempt to match the effect of the loci on elements of alcoholic consumption behavior. Once the best trait definition is identified and strong linkage is demonstrated, Wilhelmsen applies association analysis to confirm the findings and find the gene sequence changes responsible.

"The jackpot would be to figure out what aspects of alcoholism are inherited and then find the genes responsible for the disease."

A particularly cogent demonstration of this approach is a study Dr. Wilhelmsen completed in conjunction with Dr. Cindy Ehlers of the Scripps Research Institute in San Diego, California. This study investigated the heritability of substance dependence in a Southwestern California Native American population collectively known as the Mission Indians. The Mission Indian population is a geographically restricted and genetically homogeneous group with alcoholism rates as high as 65-80% for men and 37-55% for women. These statistics make exploring the risk factors for alcoholism a more straightforward endeavor.

Wilhelmsen and Ehlers recruited 885 individuals comprising 100 pedigrees. They performed high-density microsatellite genotyping (a mapping technique using a standard panel of polymorphic genetic markers) to genotype the individuals. In addition, individuals were assessed for lifetime alcohol dependence using a standard interview questionnaire (SSAGA) that diagnosed dependence based upon the DSM-III-R criteria.

Wilhelmsen and Ehlers chose to refine the definition for dependence in order to represent more restricted characteristic traits. This allowed them to investigate the effects of alleles that have large and potentially important effects on each specific alcoholic trait. Alleles are the variations in a gene that alter traits such as eye color or hair type. For alcoholism, the DSM-III-R definition of dependence was subdivided into three categories: 1) alcohol craving, 2) alcohol withdrawal syndrome, and 3) severity of alcohol use. Within the third category, subjects were selected for a) drinking in large amounts, over long periods of time, b) being drunk when they didn't want to be, c) having little or no time for anything else while drinking or recovering, and d) giving up/neglecting important activities to drink.

The results of the linkage analysis indicated that there was a possible region of importance on chromosome 5 that had not been previously identified, suggesting that their trait definition refinement strategy could lead to novel findings. In addition, the withdrawal trait mapped to three separate chromosome regions within chromosomes 6, 15, and 16. The latter two regions were previously identified using independent

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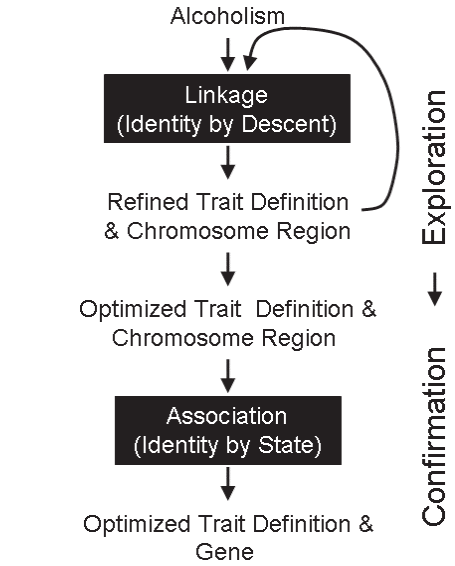

Wilhelmsen lab uses a combination of linkage analysis and association analysis to find genes that underlie the hereditary traits of alcoholism.

The Director's Column

Fulton T. Crews, Ph.D.
Director,
Bowles Center for Alcohol Studies

Genes and environment work together to manifest disease. A variety of studies indicate that alcoholism is half genetically and half environmentally determined, yet impossible to separate. Genetics alter behavior. Among dogs, labradors love the water, dachshunds love to dig, and border collies will look you in the eye as they were bred to freeze sheep with their stare. These are complex behaviors that are hard wired in genetics, much like each spider's web is genetically defined.

Human behaviors are modified by the environments people select. Choosing to frequent a bar or a church is a behavioral choice that results in a particular environment. Alcoholics often chose unhealthy environments which commonly results in more medical problems. Environments with stress, ethanol, smoking, and violence can alter gene expression in ways that permanently affect learning ability and stress response. These genetic variations can change an individual's sensation and response to alcohol. Alcohol-induced changes in gene expression likely contribute to the development of alcoholism, making alcohol a key environmental factor. Hence, the interactions of genes and environment are tangled and complex.

Good training and good genes are both necessary to become a champion. Erik Erickson, a psychoanalyst, used epigenetics to refer to stages of human developmental across the lifespan. He proposed that periods of psychological development are critical to form the authentic selfhood, not just instinctual drive and determinism. During fetal development there are key stages when alcohol-induced changes in gene expression and fetal exposure to alcohol can result in permanent damage. It is possible that the fetus is not the only developmental stage where alcohol-induced changes in genes can alter the life course. Growing evidence suggests that binge drinking in adolescence can also have a lasting effect.

Epigenetics is a term used to refer to gene modifications due to changes in gene expression rather than changes in DNA sequence. As a result of environment, epigenetic mechanisms likely contribute to adaptive changes, including alcohol-induced changes in gene expression. Understanding how genes and environment interact is going to improve the health of everyone. Sorting out which genes respond to the environment and determining what makes them unique is complicated, but is still genetics. Kirk Wilhelmsen is investigating this issue with a focus on specific phenotypes that relate to genotypes. It will not be easy, but in time we will be rewarded with better health as a result of understanding how genes are genetically and environmentally regulated. ■

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criteria of alcohol dependence (ICD 10, chromosome 16) and criteria related to late onset of alcoholism and harm avoidance personality features (chromosome 15). The concordance with previous studies suggested a more rigorous investigation of these loci might be productive in identifying genes responsible for the withdrawal phenomena. Finally, the severity of alcohol use criteria was investigated and found to map to a region of chromosome 4 that overlapped a cluster of alcohol dehydrogenase genes, a cluster of genes that form the enzymes to metabolize ethanol and are known to have alleles that contribute to protection from alcoholism in some individuals. Association analysis showed that this definition of alcohol use severity contained an important genetic variable (*ADH1B*3* allele) that influences the development of the alcoholism diagnosis in Mission Indians.

"The jackpot", said Wilhelmsen, "would be to figure out what aspects of alcoholism are inherited and then find the genes responsible for these traits. We know that there is something that is hereditary about alcoholism, but we don't know exactly what it is. Is it the ability to learn to like alcohol? Is it the ability to develop a craving for alcohol? There are numerous possibilities. We're not only trying to figure out where the genes are that lead to predisposition, we're trying to figure out what elements of alcoholism are caused by these genes."

"I'm fully expecting", Wilhelmsen adds, "to redefine alcoholism based on the underlying biological process and finding the specific traits that are inherited. If there's a gene involved, there's a molecule involved, and if this is the case, there can be a drug developed to cure it." ■

Bowles Center Receives 2005 Tharp Award

The Bowles Center for Alcohol Studies received the 2005 Tharp Award for outstanding work in the area of research and treatment of alcoholism.

James H. Tharp, a benefactor of alcoholism research, designated annual awards to support further exploration in this field. The Center shares this year's award with the Tennessee Medical Foundation and the San Diego State University Research Foundation.

Young Investigator Spotlight

Joyce Besheer, Ph.D. Discovers New Target for Alcoholism Treatment

Alcohol produces distinct stimulus effects (including good feelings) that may play a role in excessive drinking. Dr. Joyce Besheer, Research Assistant Professor at the Bowles Center, has attempted to understand the mechanisms involved in producing these stimulus properties in rodents. Test subjects have been administered moderate doses of alcohol and trained to press a particular lever for ethanol and another lever for water. Blockage of the mGluR5 subtype of metabotropic glutamate receptors using 2-methyl-6-(phenylethyl)-pyridine (MPEP) inhibits the animals' ability to discriminate ethanol correctly. The mGluR5 antagonist MPEP does not produce ethanol-like stimulus properties but appears to reduce the subjective properties of ethanol. Furthermore, Besheer's studies have found that MPEP inhibits self administration of ethanol.

The Bowles Center for Alcohol Studies

Tel. (919) 966-5678
Fax. (919) 966-5679

To become involved in our mission, call Melissa Mann at (919) 966-5678 or email melissa_mann@med.unc.edu.

For treatment information call UNC Health Care's Alcohol and Substance Abuse Program at (919) 966-6039 or (888) 457-7457.

www.med.unc.edu/alcohol

Joyce Besheer, Ph.D.

If supplementary research is able to support the observation that mGluR5 receptor antagonists have the ability to change the stimulus properties of alcohol and reduce alcohol drinking in rodents, we will be one step closer to introducing a new recovery strategy for alcoholics and alcohol abusers. "Finding that MPEP alters the stimulus properties of alcohol is very exciting and may be a possible mechanism for reduced alcohol drinking," says Besheer. "Targeting this group of receptors may be important for therapeutic development."

Dr. Besheer has been working at the Bowles Center for Alcohol Studies as a post-doctoral Research Associate under the mentorship of Dr. Clyde Hodge since July of 2002. She recently received an outstanding score on a career development award (KO1) application to NIAAA for independent studies at UNC. Dr. Besheer completed undergraduate studies at Indiana University, Bloomington, and received her Master and Doctorate degrees from the University of Nebraska, Lincoln. She has a bright future in the field of alcohol research. ■

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A. Leslie Morrow, Ph.D., Editor-in-Chief; Scott Chasse, Ph. D. and Céline van Riemsdijk, Contributing Writers

UNC Bowles Center for Alcohol Studies
CB 7178, Thurston-Bowles Building
University of North Carolina at Chapel Hill
Chapel Hill, North Carolina 27599-7178

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