Chapter

29

Endophenotypes in psychiatric genetics

Andrew C. Chen, Madhavi Rangaswamy, and Bernice Porjesz

It has been more than a half century since Nobel laureates Drs. Watson, Crick, and Wilkin discovered the structure of DNA. Since that time, rapid advancement of molecular biological technology has facilitated progress in the field of genetics, with the complete sequencing of the human genome by the Human Genome Project accomplished in 2003. It is now possible, not only to identify single gene Mendelian disorders, but also to understand "complex" disorders that are the result of multiple small gene effects that predispose individuals to these disorders, as they interact with environmental factors. As we try to envision the future direction of human genetics research, particularly the genetics of complex genetic diseases such as the majority of psychiatric diseases, it would be helpful to briefly review some important milestones for the development of genetics in terms of *phenotypes* before the era of molecular biology.

A few years after the term "genetics" was first introduced by William Bateson in 1902, the Danish botanist Wilhelm Johanssen attempted to provide the clarifying distinction we now take for granted between the concepts of "genotype" and "phenotype". He also introduced the word "gene" in 1909, a half century before the discovery of the structure of the genetic molecule, DNA. Through his research on self-fertilized lines of beans, Johanssen found that the phenotype is often an imperfect indicator of the genotype: the same genotype may give rise to a wide range of phenotypes. Similarly, the same phenotype may have arisen from different genotypes. This important observation was contradictory to the genetic theory at that time, which endorsed the idea that there was a one-to-one correlation between genotype and its "consequential" phenotype. About the same time, Herman Nilsson-Ehle, a Swedish expert in plant breeding and genetics, provided evidence for genetic *and* nongenetic contributions to a continuous phenotype on the basis of observations of seed colors in crosses of oats and wheat [1]. As we now know, a genotype is the composition of DNA sequences; in contrast, a phenotype represents observable characteristics of an organism, and is the joint product of both genotypic *and* environmental influences. In genetic diseases that are transmitted through the classic or Mendelian model, genotypes are usually indicative of phenotypes in terms of the presence or absence of the disease. However, this certain correlation between genotypes and phenotypes does not exist in complex genetic diseases.

Recently, there has been a surge of interest in the use of endophenotypes in psychiatric research, although the concept was first introduced to the field of psychiatry by Gottesman and Shields more than three decades ago [2]. This has been driven by concerns about the limited success and relatively poor reproducibility of the current psychiatric genetic research approach, and the fact that current psychiatric diagnostic systems, DSM or ICD, are primarily based on phenomenology and lacking of justification by etiology. Psychiatry's classification systems describe heterogeneous disorders [3]. The brain is the most complex of all organs. It is subject to complex interactions not only among genes, proteins, cells, and circuits of cells, but also across individuals and their changing experiences [4]. As such, the phenotypic output from the brain, i.e. behavior, is not simply a sum of all its parts. Therefore, it would be a more reasonable approach to apply more optimally reduced measures of neuropsychiatric functioning than a behavioral complex, the diagnosis, in studies of the biological and genetic components of psychiatric disorders. It has been suggested that ideally, molecular genetic studies should not be

Principles of Psychiatric Genetics, eds John I. Nurnberger, Jr. and Wade H. Berrettini. Published by Cambridge University Press.

performed on psychiatric diagnoses alone, which reflect distal and variable effects of genes, but on quantitative neurobiological measures or markers that reflect more proximal effects of genes involved in the genetic predisposition for developing psychiatric disorders [5].

The endophenotype concept

An endophenotype is typically an unobserved phenotype, such as metabolite level, cell or organ activity, biosignal, or other "biomarker", that is thought to contribute to the etiology of a visible phenotype or disease susceptibility. It is associated with a disease but can be measured independently of disease status. Gottesman and Gould have defined endophenotype as "measurable components unseen by the unaided eye along the pathway between disease and proximal genotype" [6]. In their writings summarizing genetic theories in schizophrenia 30 years ago, Gottesman and Shields described "endophenotypes" as internal phenotypes that lie on the pathway between genes and disease, and can be observed only by a "biochemical test or microscopic examination" [2]. These quantitative biological markers serve as covariates that correlate with the main trait of interest (diagnosis) and serve to better define that trait or its underlying genetic mechanism. The term "endophenotypes" was adapted from a 1966 paper explaining concepts in evolution and insect biology by John and Lewis [7]; they wrote that the geographical distribution of grasshoppers was a function of some feature not apparent in their "exophenotypes" that are obvious and external. In contrast, these features were "microscopic and internal", hence the term, "endophenotype". Thus, the term endophenotype appears to fit the needs of psychiatric genetics, and this concept bridges the gap between the gene and the complexity of psychiatric disease processes. Typically, endophenotypes are quantitative traits that can be measured in a general population. That is, the traits are present in individuals who have manifested the symptoms of the disease as well as in those who do not have the disease, including those at risk prior to the onset of the disease.

Generally speaking, the utility of the endophenotypes is relevant for two types of studies. First, as originally proposed [2, 6], they are used to aid in the discovery of novel genes that are associated with the disorders. Second, an increasingly popular method is to apply endophenotypes to study the functional consequences of risk alleles.

Characteristics useful for the identification of endophenotypes in psychiatric genetics

There have been a number of attempts to devise criteria to define the optimal characteristics of an endophenotype [6, 8–10]. As the diagnostic categories of psychiatric disorders in use today were initially formulated in the late nineteenth and early twentieth centuries by a small number of psychiatrists who relied on perceived similarities in behavioral syndromes and clinical outcomes, we should be aware that such categories reflected only observable behaviors rather than dysfunctions in distinct anatomical substrates or patho-physiological processes. Any given disorders with singular labels, such as schizophrenia or attention-deficit hyperactivity disorder (ADHD), are probably better thought of as a heterogeneous group of dysfunctions whose final pathways eventually lead to similarity in symptoms, course of illness, and other clinical features. For endophenotypes to be useful in psychiatric genetic analyses, they should have several properties [6, 11]. We summarize the six properties below. The first two are most necessary for any endophenotype to be useful in medical genetics. The last four, although not strictly necessary, are properties that can aid in the successful genetic dissection of complex behaviors.

The endophenotype is heritable

Complex traits or phenotypes are the outcome of an interaction between genetic and environmental factors. One of the most important criteria defining an endophenotype is that it be a heritable trait. For endophenotypes to aid in the genetic dissection of complex traits, there must be some evidence of heritability of the phenotype. It is not very useful to link a trait to genes if genetic factors do not contribute to the individual differences in the trait, i.e. when it is largely influenced by environmental factors. However, it should be kept in mind that using endophenotypes of relatively low heritability may still be important, particularly when there is a potential for gene × environment interaction. As environmental effects may be crucial in altering the effects of even

highly heritable disorders, these endophenotypes can also aid in the development of pharmacological or behavioral treatments and interventions.

The endophenotype is associated with the disorder

There should be reasonable evidence that the endophenotype is associated with the pathophysiology of the illness. Ideally, it should be a part of the causal pathway from gene to disorder rather than effects (sequelae) of disorders or their treatment. This (to be associated with the causes of the disorders) is important when the endophenotype is being used to highlight the risk genes, and relatively less important when the endophenotype is applied to study the functional consequences of known risk alleles.

The endophenotype is present in both affected and nonaffected individuals, and it varies continuously in the general population

Although the diagnosis of mental disorders is dichotomous, i.e. whether a certain disorder is present or not, it is unlikely that common mental disorders vary in a discrete manner between individuals. Statistical analyses of continuous traits that are artificially categorized into discrete groups have substantially less power than analyses conducted on the original continuous scale. Therefore, it would be more powerful to analyze endophenotypes that can be measured on continuous scales. This also highlights another important advantage of analyzing endophenotypes that vary continuously in the nonaffected population because it can simplify the sampling process and reduce ascertainment biases in population-based studies [12].

The endophenotype is primarily state-independent

As mentioned, to be useful for genetic analysis, the endophenotype should be present in both affected and unaffected individuals. To aid in the discovery of novel genes that are associated with the disorders, the endophenotype should also manifest in an individual at a similar quantitative level regardless of whether or not the illness is active. It should demonstrate adequate test–retest stability and reliability.

Within families, endophenotype and illness co-segregate

Affected members of families manifest the endophenotypic marker significantly more than unaffected members of the families.

The endophenotype found in affected family members is found in nonaffected family members at a higher rate, or at a higher level, than in the general population

This will allow small to moderate effect sizes between biological relatives of affected patients and community controls to be observed more easily [13].

Application of endophenotypes in psychiatric genetics

A fundamental assumption for the application of endophenotype in psychiatric genetic research is that the number of genes required to produce variations in these traits is fewer than those involved in producing a psychiatric diagnostic entity, which may reflect a more distal end-point of the combined contributions from multiple genetic and environmental factors, as well as the interactions among them. Quantitative neurobiological measures of risk as endophenotypes are closer to gene action involved in the predisposition for the disorder. Therefore, in searching for genetic variations that are involved in complex (non-Mendelian) psychiatric diseases, endophenotypes provide a more powerful strategy than the dichotomous diagnosis of the disease [6, 14, 15]. In psychiatry, a number of attempts have been made to develop and determine the feasibility of candidate endophenotypes. Although few candidates have met all the criteria listed earlier, some linkage and association studies using the endophenotype strategy have had moderate success.

This chapter is organized into two main sections. In the first part of this chapter, we summarize representative published studies that have successfully used various endophenotypes to find candidate genes involved in several psychiatric disorders, including schizophrenia, mood disorders, Alzheimer's disease (AD), ADHD, and suicidal behavior. The second half of this chapter focuses on alcoholism, and more fully illustrates the successful use of the endophenotype

strategy in the Collaborative Study on the Genetics of Alcoholism (COGA) project, where we have used brain oscillations as endophenotypes in the identification and understanding of genes involved in alcoholism and related disinhibitory disorders.

Sensory motor gating as endophenotypes for schizophrenia

Deficits in sensory motor gating are consistent neuropsychological findings in individuals suffering from schizophrenia [16]. The hypothesized association has face validity primarily on the basis of patients' reports that they may have difficulty filtering information from multiple sources that occur in everyday life [16–18]. Neurophysiological tests, including assessments of P50 suppression and pre-pulse inhibition of the startle response, have been developed to discern efficiencies in these capabilities.

In tests of pre-pulse inhibition, startling sensory stimuli (e.g. loud noise, bright light) are used to elicit an unconditional reflexive startle response in individuals. If a weaker pre-stimulus is provided before the startling stimulus, the subsequent startle response is generally diminished. A relatively reproducible finding is that this diminution of the second response is attenuated in patients with schizophrenia [16, 17]. Of note, abnormal pre-pulse inhibition is not specific to schizophrenia; studies have identified this abnormality in obsessive–compulsive disorder [19] and Huntington's disease [20].

The P50 suppression test uses two auditory stimuli presented at 500 ms intervals. Event-related potentials (ERPs) for both stimuli are measured by electroencephalogram (EEG). In normal individuals, the ERP to the second stimulus is of lower amplitude than the first. However, suppression of P50 amplitude is compromised in patients with schizophrenia [16, 17, 21, 22] as well as in their unaffected first-degree relatives [22–24]. Studies on the heritability of P50 suppression have strongly suggested that the variation in P50 is under the influence of genetic factors and that P50 suppression is a good candidate endophenotype [25]. Freedman and colleagues [26] used P50 suppression to identify a potential susceptibility locus for schizophrenia on chromosome 15, a chromosomal region where the *CHRNA7* gene encoding the alpha-7 nicotinic acetylcholine receptor resides. Linkage disequilibrium in this region [27] has subsequently been reported, and variations in the promoter region of the *CHRNA7* gene have been found to be associated with schizophrenia and/or P50 suppression abnormalities [27, 28].

Eye-tracking dysfunction as endophenotypes for schizophrenia and bipolar disorder

Eye movements are generally of two forms: saccadic (brief and extremely rapid movements) and smooth ("smooth pursuit" eye movements occur only when the subject is following an object moving at a constant velocity, such as a pendulum or bright dot on a computer monitor). Initiation and maintenance of smooth pursuit eye movements (SPEMs) involve integration of functions of the prefrontal cortex frontal eye fields, visual and vestibular circuitry, thalamus, and cerebellum, as well as the muscles and neural circuitry directly responsible for eye movement [29]. Studies have found that patients with schizophrenia and their unaffected relatives have increased rate of deficiencies in SPEMs [30, 31]. Similar results have also been reported in bipolar disorder [31, 32]. Studies have reported evidence for linkage of SPEM phenotypes to chromosome 6p23–21 [33, 34]; the region harbors two genes associated with risk of schizophrenia, *ATXN1* (*SCA1*) and *NOTCH4* [32].

Neurocognitive endophenotypes for schizophrenia and ADHD

Family and twin studies have suggested moderate heritability of working memory deficits in schizophrenia [35–37]. Recently, researchers such as the Consortium on the Genetics of Schizophrenia (COGS), a seven-site collaboration that examines the genetic architecture of quantitative endophenotypes in families with schizophrenia, have attempted to select neurocognitive tasks as endophenotypic measures in genetic studies [38, 39]. The COGS neurocognitive assessment includes measures of attention, verbal memory, working memory, and a computerized neurocognitive battery that also includes facial processing tasks [40].

A study of Finnish twins [41] using the sum of performance scores on four neuropsychological tests as an endophenotype suggested linkage and association to a region of chromosome 1q, a region previously suggested in traditional linkage studies of schizophrenia [42]. Studies [43] have also shown an association of poorer performance on a working

memory task with the gene encoding the enzyme catechol-*O*-methyltransferase (COMT) located at chromosome 22q and increased risk for developing schizophrenia. This chromosomal region has been reported to be linked to both schizophrenia and bipolar disorder and overlaps with a deletion that has been associated with velo-cardio-facial syndrome (DiGeorge syndrome) and schizophrenia [44]. A functional polymorphism, Val158Met, which causes a valine to methionine mutation at peptide 158 in the gene encoding COMT, results in four-fold decreased activity in the rarer Met allele, hence preferentially increasing prefrontal extra-synaptic dopamine, because COMT provides the major clearing step for dopamine released from the synapse in this region [45]. The more common Val allele is associated with less efficient cognitive processing by the brain, and is transmitted at a higher rate than the Met allele to patients with schizophrenia than to their nonaffected siblings [46].

Similarly, many neurocognitive measures of executive functions, for example, Attention Network Task, Continuous Performance Test, Matching Familiar Figures Test, Span of Apprehension Test, Spontaneous Selective Attention Task, and Wisconsin Card Sorting Test, have been proposed as endophenotypes for ADHD [47, 48]. Studies have shown that deficits in neurocognition are a correlate of ADHD and show preliminary evidence of heritability and association with relevant candidate genes. Nonetheless, studies that have assessed the familial and genetic overlap of neurocognitive impairments with ADHD have yielded inconsistent results. In order for executive function deficits to be used as an endophenotype for ADHD, more studies with greater attention to the neurocognitive heterogeneity of this disorder and to the precision of measurement of the neuropsychological tests are required [49].

Neuroimaging endophenotypes

Another powerful and rapidly evolving technique used in psychiatric genetic studies is functional magnetic resonance imaging (fMRI), which combines the neurocognitive tasks with the anatomical location of brain activities. Studies have demonstrated that the more common Val allele of the functional polymorphism Val158Met in the *COMT* gene is associated with less efficient cognitive processing by the brain, and that more blood flow in the prefrontal cortex (PFC) was needed to accomplish the same task in the fMRI assessment, suggesting that activation of the dorsolateral prefrontal cortex is less efficient in those subjects [50]. This inefficiency was observed in schizophrenic subjects as well as unaffected siblings of schizophrenic patients [51].

In agreement with this discovery, variation in *COMT* also modulates other PFC-dependent neuropsychological performance [45] and the cortical response to amphetamine, which increases synaptic dopamine [52]. The latter finding suggests that the *COMT* genotype links prefrontal dopamine stimulation and neuronal activities, in which homozygotes for the Val-encoding allele presumably possesses less synaptic dopamine due to maximal COMT activity than the Met allele carriers. Additional evidence for this comes from a positron emission tomography (PET) study [53] showing that the *COMT* genotype has an impact on the prefrontal regulation of midbrain dopamine synthesis.

In addition to fMRI, phenotypes measured by anatomical imaging such as structural MRI have also been proposed as endophenotypes for some psychiatric disorders. For example, reduction in orbitofrontal volume in ADHD [54], decreased gray matter in insular cortex, and temporal lobe gray matter abnormalities in schizophrenia [55]. Recent studies using diffusion tensor imaging (DTI) and magnetization transfer ratio (MTR) protocols have begun to shed more light on the white matter deficits among schizophrenia patients, and studies on their heritability (including their potential as endophenotypes) are under way [55, 56].

Neurochemical metabolites as endophenotypes

In addition to soft neurological signs and neurocognitive performance, many studies have focused on neurochemicals, neurohormonal substances, and their metabolites in search of candidate endophenotypes for psychiatric disorders. Here we use a few recent studies in AD and suicide as examples.

Aggregation and deposition of amyloid beta (Aβ) in the brain is thought to be central to the pathogenesis of Alzheimer's disease (AD). Recent studies suggest that cerebrospinal fluid (CSF) Aβ levels are strongly correlated with AD status and progression. Using CSF Aβ levels as a phenotype for AD, a recent study has identified a DNA sequence variation in the

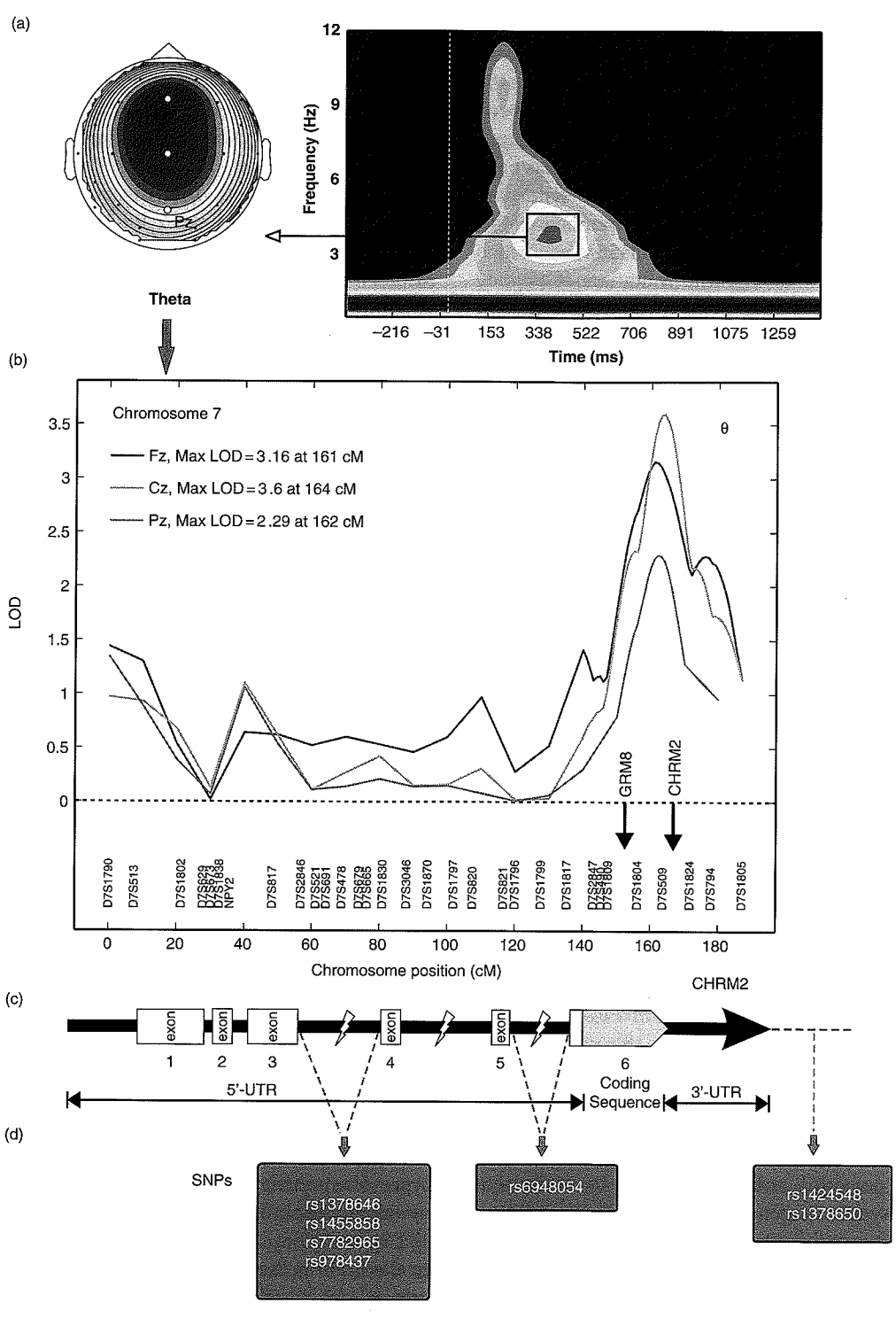

Figure 29.1 Illustration of endophenotype strategy: from neurocognitive endophenotypes to genes. (a) Endophenotype (Theta oscillation), Theta (θ, 3–7 Hz) event-related oscillations (EROs) underlying the processing of target stimuli during P3 production (300–700 ms) in a visual oddball task as seen in this time-frequency representation (right panel). Head plot (left) displays scalp topography of theta ERO power.

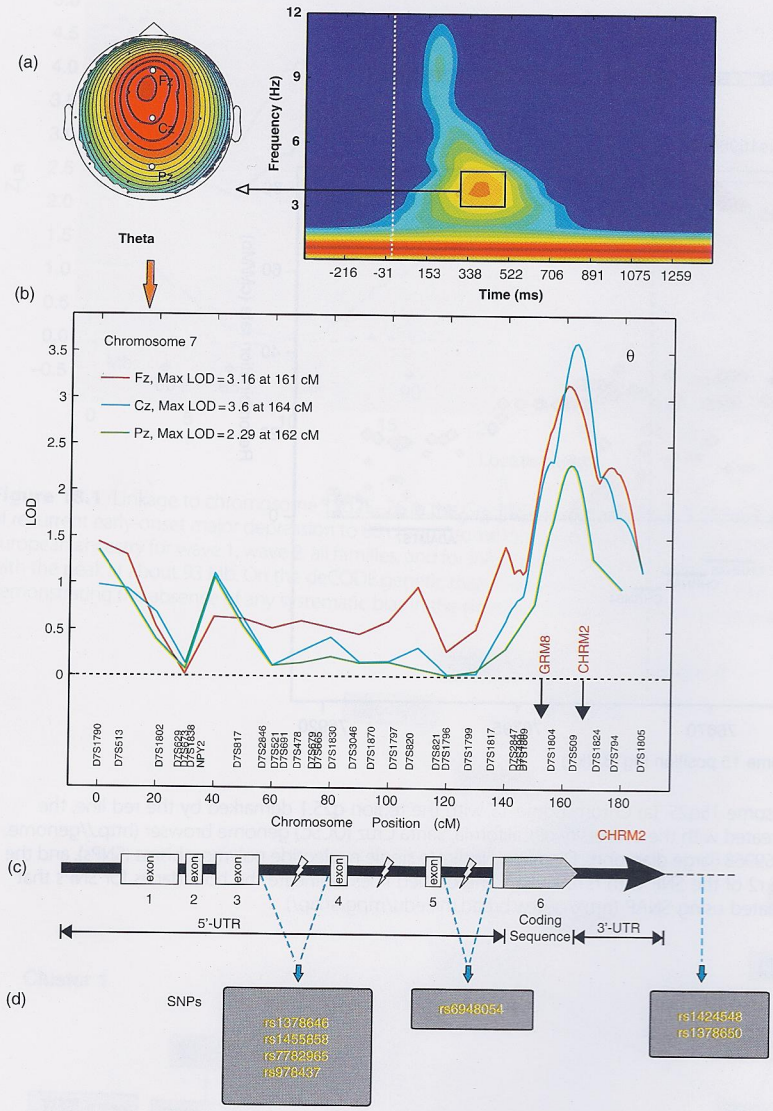

Figure 29.1 Illustration of endophenotype strategy: from neurocognitive endophenotypes to genes. (a) Endophenotype (Theta oscillation), Theta (θ, 3–7 Hz) event-related oscillations (EROs) underlying the processing of target stimuli during P3 production (300–700 ms) in a visual oddball task as seen in this time-frequency representation (right panel). Head plot (left) displays scalp topography of theta ERO power. (Theta EROs are correlates of impaired cognitive brain processes in alcoholism and risk.). (b) Genetic linkage analysis scans the entire genome to assess chromosomal regions that contain polymorphic genetic markers that are linked to a quantitative trait (theta ERO power) within families. This is a logarithm of odds (LOD) score plot of linkage for theta ERO power at frontal (Fz), central (Cz), and parietal (Pz) electrodes on chromosome 7 with a significant linkage peak that contains 2 candidate genes under the linkage peak – *CHRM2* and *GRM8*. (c) Candidate gene studies focus on genes underlying significant linkage peaks and/or genes with relevant biological significance. This is a schematic diagram of the *CHRM2* gene indicating its coding region (gray) and exons. (d) Genetic association tests are performed for each single nucleotide polymorphism (SNP) in the candidate gene and the trait variable. SNPs are identified within and flanking the candidate *CHRM2* gene (exons and introns). SNPs with significant associations (gray boxes) with the endophenotype indicate loci of interest. (The right-most box with two SNPs lies in the region beyond the 3′ UTR of the gene.)

presenilin 1 (*PSEN1*) gene that is a novel disease-causing mutation in clinically characterized research subjects with extreme values of CSF [57].

Another example is cortisol response to psychosocial stress with regard to suicidal behavior. Disturbances in hypothalamic-pituitary-adrenal (HPA) axis function have been observed in suicide attempters using various indices, including CSF corticotrophin releasing hormone (CRH), cortisol levels following dexamethasone challenge, and urinary cortisol [58, 59]. Twin studies of cortisol levels in blood, urine, and saliva estimated heritability to be approximately 60% [60]. A twin study of cortisol response to psychosocial stress (Trier Social Stress Test [TSST]) estimated heritability of cortisol response with repetition of the stressor to be between 56 and 97% [61]. Several polymorphisms that have been shown to be associated with cortisol response to TSST include the mineralocorticoid and glucocorticoid receptor genes [62], the *5HTTLPR* [63], and the γ-aminobutyric acid (GABA)-A α-6 receptor gene [64]. Studies are necessary to establish if the same deficits in cortisol response are more frequent in unaffected relatives of suicidal individuals compared with the general population.

Neurocognitive phenotypes in alcoholism and related disorders: brain oscillations as endophenotypes

In this section, we more fully illustrate the endophenotype approach – from endophenotype to genes, using brain oscillations as endophenotypes in the search for genes predisposing to alcoholism and related disinhibitory disorders in the COGA project (Figure 29.1). Alcoholism is a common complex (non-Mendelian) disorder with contributions from both genetic and environmental influences and their interactions. Recent evidence suggests that alcohol dependence is part of a spectrum of disinhibitory disorders, which include externalizing and other substance use disorders. Many of the same genetic risk factors are postulated to underlie these disinhibitory co-occurring disorders which are explained by a common underlying genetic liability involving impulse control [65]. These findings are in keeping with electrophysiological findings reflecting a similar electrophysiological profile in these related disinhibitory disorders [15]. Brain dysfunction is likely to be involved in a genetic predisposition to develop alcoholism and other psychiatric disorders, and neuroelectric events may serve as excellent biological markers or endophenotypes.

Brain oscillations as endophenotypes

Brain oscillations provide a rich source of potentially useful endophenotypes for psychiatric genetics, as they represent important neural correlates of information processing and cognition in humans. These oscillations are dynamic indices of the millisecond by millisecond balance between excitation and inhibition in brain neural networks and have exquisite temporal resolution. They reflect ensembles of neurons firing in synchrony and represent the basic mechanism of neural communication. High frequency (beta [12–28 Hz], gamma [28+ Hz]) synchronizations are involved in short-range communication, while low frequencies (delta [1–3 Hz], theta [4–7 Hz], alpha [8–12 Hz]) are involved in longer range communication between brain areas [66]. Brain oscillations represent traits less complex and more proximal to gene function than either diagnostic labels or traditional cognitive measures. Therefore, these oscillations may be utilized as phenotypes of cognition, and as valuable endophenotypes for the understanding of some complex genetic brain disorders. Several brain oscillations meet criteria for endophenotypes (see above); they are highly heritable: delta 76%, theta 89%, alpha 89%, and beta 86% [67].

Figure 29.1 (*cont.*) (Theta EROs are correlates of impaired cognitive brain processes in alcoholism and risk.). (b) Genetic linkage analysis scans the entire genome to assess chromosomal regions that contain polymorphic genetic markers that are linked to a quantitative trait (theta ERO power) within families. This is a logarithm of odds (LOD) score plot of linkage for theta ERO power at frontal (Fz), central (Cz), and parietal (Pz) electrodes on chromosome 7 with a significant linkage peak that contains 2 candidate genes under the linkage peak – *CHRM2* and *GRM8*. (c) Candidate gene studies focus on genes underlying significant linkage peaks and/or genes with relevant biological significance. This is a schematic diagram of the *CHRM2* gene indicating its coding region (gray) and exons. (d) Genetic association tests are performed for each single nucleotide polymorphism (SNP) in the candidate gene and the trait variable. SNPs are identified within and flanking the candidate *CHRM2* gene (exons and introns). SNPs with significant associations (gray boxes) with the endophenotype indicate loci of interest. (The rightmost box with two SNPs lies in the region beyond the 3′ UTR of the gene.) See plate section for color version.

In our strategy for finding susceptibility genes for alcohol dependence and related disorders, we select brain oscillations that not only differentiate between alcoholics and controls, but also between high risk offspring of alcoholics and controls, to be sure that we are selecting "trait" rather than "state" measures that meet criteria for good endophenotypes. COGA is a genetic study of densely affected families ascertained through affected probands in treatment, where there were at least three affected (DSM III-R and Feighner definite for alcohol dependence) first-degree relatives. We focused on neuroelectric endophenotypes (such as resting eyes-closed EEG [e.g. power and coherence], ERPs [e.g. the P3 component], and event-related oscillations [EROs] [e.g. theta and delta event-related oscillations]) recorded during sensory and cognitive tasks. As will be shown in this chapter, we have successfully used this approach with brain oscillations as endophenotypes first to target chromosomal regions using linkage analysis, and secondly identifying potential candidate genes that may be involved in both brain function and diagnosis of alcohol dependence and related disorders contained in the chromosomal regions under that linkage peak. These genes are then more closely investigated using association analyses (Figure 29.1).

Resting EEG beta power: *GABRA2* gene

EEG signatures of the resting state of the brain have revealed characteristic patterns in individuals with alcoholism and also those with high risk for developing alcoholism and related spectrum of externalizing conditions [15]. Across most studies published in this field, increased beta power in the EEG has emerged as one such important feature; it is noted in affected [68–73] and high risk offspring of alcoholics [74, 75], including a large sample of offspring of alcoholics at high risk from the COGA project [76]. Increased resting beta power has been reported at frontal leads in those who also have a diagnosis of antisocial personality disorder (ASP) [77]. Also, increased beta power may be associated with increased vulnerability, as female high risk subjects with a larger number of affected first-degree relatives displayed significantly elevated beta power compared to those with just one affected parent [72]. Thus evidence of elevated beta power provides strong support for the excitation–inhibition imbalance model proposed to underlie the predisposition to alcohol dependence [78].

As increased resting beta power is already observed in offspring before the onset of alcohol dependence, it is considered to be a trait rather than a state measure. Significant linkage and linkage disequilibrium between the EEG beta frequency and a γ-aminobutyric acid ($GABA_A$) receptor gene on chromosome 4 has been reported by COGA (Figure 29.2) [79]. With the use of multiple single nucleotide polymorphisms (SNPs) across this cluster of $GABA_A$ receptor genes on chromosome 4, that includes *GABRA2, GABRA4, GABRB1*, and *GABRG1*, we were able to specifically identify that it was variations only in the *GABRA2* receptor gene that accounts for the linkage /linkage disequilibrium findings with the beta frequency. Thus, variations in *GABRA2* (the gene encoding the α-2 subunit of the $GABA_A$ receptor) affect brain oscillations and are directly involved in the level of neural excitability (balance between excitation and inhibition). There is a strong relationship between the most significant SNP (rs279836) in the *GABRA2* receptor gene and beta EEG power. Of note individuals who are homozygous for the rarer genotype (15%) of this SNP have significantly increased EEG beta compared to individuals with all other genotypes, indicating underlying central nervous system (CNS) disinhibition. The same *GABRA2* gene associated with the EEG beta endophenotype was subsequently found to be associated with DSM-IV diagnosis of alcohol dependence [80], substance dependence [81], ASP [82], and childhood conduct disorder [83]. The association between *GABRA2* and alcohol dependence has been replicated by a number of groups throughout the world [84–89].

Fast synaptic inhibition in the mammalian CNS is mediated largely by activation of $GABA_A$ receptors [90]. $GABA_A$ actions are a fundamental requirement for both gamma and beta oscillations to occur, and blockade of these receptors results in loss of synchronization [91]. Although the recording of electrical oscillations from a neural population reflects the firing of multiple excitatory pyramidal cells, the mechanism underlying beta and gamma oscillations depends on the firing patterns of a network of inhibitory interneurons [92, 93], that are gated by their mutually induced $GABA_A$ action [94].

These genetic findings relating EEG beta and the *GABRA2* gene suggests that variations in the *GABRA2* gene affect the inhibitory tone and thus affect the level of neural excitability, which may underlie the predisposition to develop alcohol dependence and related

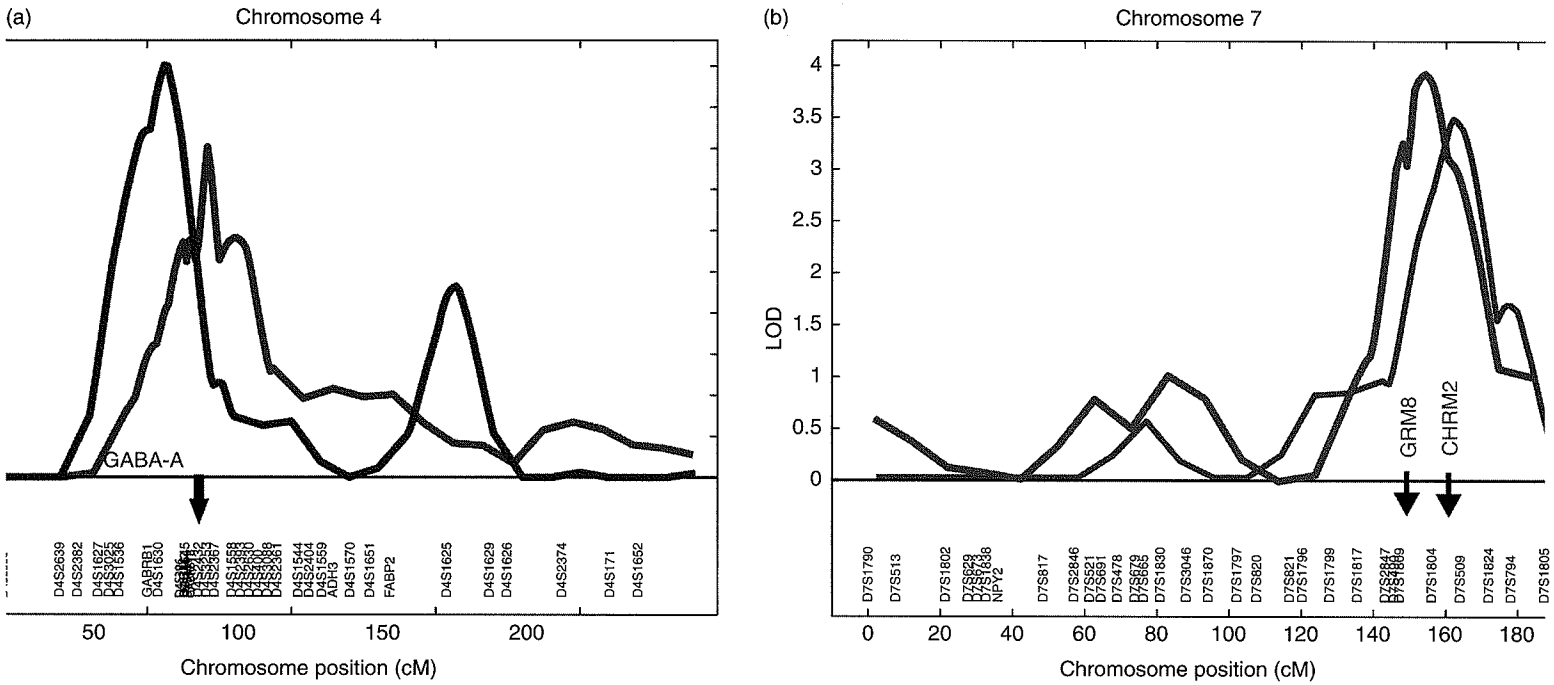

Figure 29.2 (a) Linkage plots showing maximum logarithm of odds (LOD) scores with significant linkage peaks over the $GABA_A$ receptor gene cluster on chromosome 4 with two resting EEG phenotypes. Red trace: Resting EEG beta band power – beta 2 (16.5–20.0 Hz). The dataset consists of 1553 individuals from 250 families. Green trace: Resting EEG high theta band (6–7 Hz) interhemispheric coherence at parieto-occipital bipolar pairs of electrodes (P4_O2-P3_O1). The dataset consists of 1312 individuals from 251 families.
(b) Linkage plot showing maximum LOD scores with significant linkage peaks on chromosome 7, over the region harboring two candidate genes: a cholinergic muscarinic receptor gene (*CHRM2*) and a glutamate receptor gene (*GRM8*). Blue trace: the central midline theta (4–5 Hz) ERO band power (between 300–700 ms, P3 latency window for visual target case during visual oddball task) on chromosome 7. The dataset consists of 1337 individuals from 253 families; Green trace: Resting EEG theta band (6–7 Hz) interhemispheric coherence at centro-parietal bipolar pairs of electrodes (C4_P4-C3_P3) on chromosome 7. The dataset consists of 1312 individuals from 251 families. See plate section for color version.

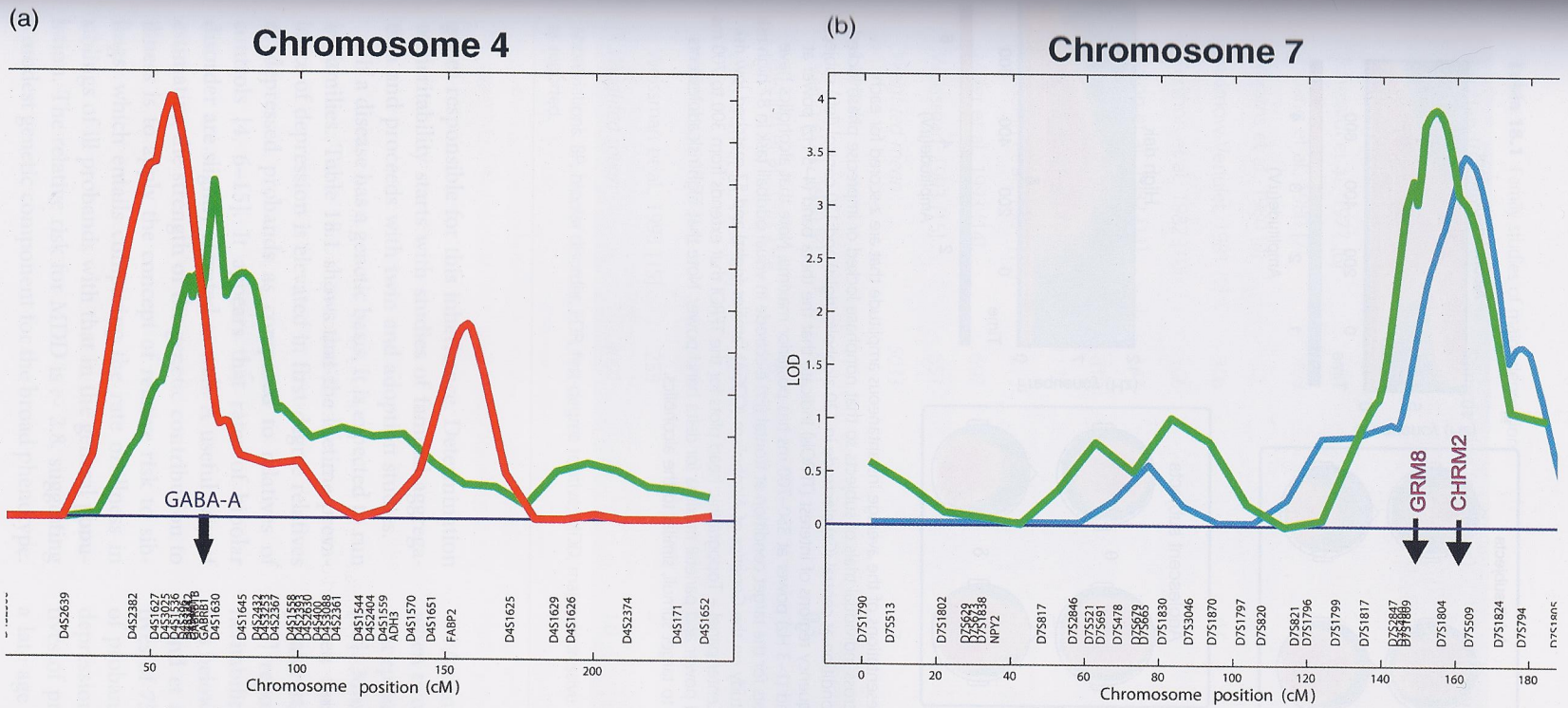

Figure 29.2 (a) Linkage plots showing maximum logarithm of odds (LOD) scores with significant linkage peaks over the $GABA_A$ receptor gene cluster on chromosome 4 with two resting EEG phenotypes. Red trace: Resting EEG beta band power – beta 2 (16.5–20.0 Hz). The dataset consists of 1553 individuals from 250 families. Green trace: Resting EEG high theta band (6–7 Hz) interhemispheric coherence at parieto-occipital bipolar pairs of electrodes (P4_O2-P3_O1). The dataset consists of 1312 individuals from 251 families. (b) Linkage plot showing maximum LOD scores with significant linkage peaks on chromosome 7, over the region harboring two candidate genes: a cholinergic muscarinic receptor gene (*CHRM2*) and a glutamate receptor gene (*GRM8*). Blue trace: the central midline theta (4–5 Hz) ERO band power (between 300–700 ms, P3 latency window for visual target case during visual oddball task) on chromosome 7. The dataset consists of 1337 individuals from 253 families; Green trace: Resting EEG theta band (6–7 Hz) interhemispheric coherence at centro-parietal bipolar pairs of electrodes (C4_P4-C3_P3) on chromosome 7. The dataset consists of 1312 individuals from 251 families.

disorders. Alcoholics and offspring at high risk manifest increased power in EEG beta oscillations, suggesting an imbalance between excitation–inhibition (CNS disinhibition). This provides a biological hypothesis relating the underlying CNS disinhibition to the genetic risk for alcohol dependence and related disorders [78]. The involvement of the GABAergic system in alcoholism is supported by neuroimaging studies, which report specific deficits in GABA benzodiazepine receptor function in the brains of alcoholics [95–97] and individuals at risk [98]. Taken together, these findings suggest GABA deficits probably contribute to a state of hyperexcitability in the brains of alcoholics and individuals at risk, and this may underlie the predisposition to develop alcoholism.

Interhemispheric theta coherence: *GABRA2* and *CHRM2* genes

Another EEG measure that provides a good endophenotype for genetic study is EEG coherence – a measure of cortical synchronization in neural networks, indexing communication in populations of neurons. Recent studies have suggested a significant role for theta frequency coherence in normal and aberrant thalamocortical interactions [99, 100]. Impairments in neural synchrony have been reported in several psychiatric conditions, including alcohol dependence. There is evidence in the literature that not only do alcoholics manifest differences in EEG power in specific frequency bands, but they also manifest increased interhemispheric coherence [101, 102]. It has been reported that bilateral intrahemispheric coherences in alpha and beta frequency bands were increased in both long-term abstinent and nonabstinent alcoholics compared to controls [103], particularly for alpha when depressiveness was included as a covariate; there was no effect of length of abstinence on these findings. In our laboratory we have observed significant increases in resting EEG interhemispheric high theta (6–7 Hz) coherence in alcohol-dependent subjects when compared to normal controls, particularly posteriorly at parietal-occipital and centroparietal regions [Rangaswamy et al., unpublished]. Peak theta band coherence was highest for posterior electrode pairs in alcohol dependent subjects, displaying a shift from a more frontocentral prominence as seen in normal control subjects. Hence, this increased EEG coherence (cortical synchronization) observed in alcoholics may be indicative of altered functional thalamocortical and cortico-cortical connectivity.

We conducted a whole genome linkage analysis in COGA using the high theta coherence at parietal-occipital leads as the phenotype. Highly significant linkage was found on chromosome 4 in the same region spotlighted by the EEG beta power phenotype [104; Rangaswamy et al., unpublished]. Family-based association analyses with the cluster of $GABA_A$ receptor genes under this linkage peak revealed strong association with a large number of SNPs (several at $p < 0.001$) genotyped in *GABRA2* for the Caucasian-only subset, and not with other genes in the cluster (Figure 29.2).

Another earlier study [105] reports that three exonic variants of the gene encoding the human $GABA_B$ receptor on chromosome 6 modify scalp-recorded EEG coherence (cortical synchronization). Parietotemporal coherence showed statistical significance associated with exon 7, suggesting that this exon may be functionally meaningful and impact on cortical EEG oscillations.

These results suggest that *GABRA2* may indeed influence susceptibility to alcohol and drug dependence, not just by modulating level of neural excitation, but also by influencing functional connectivity of interhemispheric networks. In their model of thalamocortical dysrhythmia, Linas and co-workers have proposed that the enhanced low-frequency (theta) oscillations in the thalamocortical module can affect the lateral inhibitory drive in the cortex and eventually result in high frequency coherent activation of cortical module [106]. This is particularly significant in light of our genetic findings where two resting state electrophysiological signatures – beta power (high frequency activity associated with arousal) and theta coherence (low frequency synchrony) are both linked to the same $GABA_A$ receptor gene.

The same interhemispheric high theta coherence phenotype at centroparietal leads indicated significant linkage on chromosome 7 and significant associations with a muscarinic acetylcholine receptor (M2) gene (*CHRM2*), underlying the linkage peak using family-based association tests [104] (Figure 29.2). Significant linkage and association for evoked theta band responses at the same *CHRM2* gene have been previously reported in COGA [107, 108] (see below).

Both GABAergic and cholinergic systems interact significantly in the functions of local inhibitory circuits, thus affecting network functions and influencing cortical synchronization. Increased GABAergic

inhibition is likely to be a mechanism underlying impaired synaptic plasticity observed with M2 knockout mice, who demonstrate impaired behavioral flexibility and memory deficits [109]. A recent study suggested that M2 receptor activation produces a presynaptic inhibition of GABA release by long-range inhibitory neurons of the perirhinal cortex projecting to the entorhinal cortex [110].

Theta and delta event-related oscillations underlying P3 during target detection: *CHRM2* and *GRM8* genes

One of the most consistent robust findings in the literature is the reduced P3 amplitude in alcoholics and in offspring at high risk prior to alcohol exposure [111] (for review see [15]), providing a good endophenotype for genetic studies. However, the P3 component is not a unitary phenomenon, but emanates from multiple sources in the brain with contributions from frontal cortex (including anterior cingulate), amygdala and hippocampus [112–115]. Low P3 amplitudes coupled with weaker and less well-organized sources in alcoholics and offspring at risk suggest inefficient allocation of resources during neural processing. This undifferentiated neurophysiological pattern suggests a level of cortical disinhibition in alcoholics and individuals at risk. The low P3 amplitude is not only observed in abstinent alcoholics and offspring of alcoholics, but is also present in various disinhibitory conditions, such as substance abuse, ASP, conduct disorder, and ADHD. Moreover, individuals with low P3 amplitudes manifest a significantly higher incidence of externalizing disorders and disinhibitory traits than those with high P3 amplitudes [15].

More recently, event-related activity recorded during cognitive tasks, including paradigms eliciting the P3 component, has been examined in the frequency domain (i.e. EROs). Several studies have demonstrated that P3 responses are primarily the outcome of theta and delta EROs elicited during cognitive processing of stimuli [116–120]. Topographically, delta ERO power peaks at the posterior region, while the theta power peak is located in the frontocentral region [119]; theta oscillations also contribute strongly to N2 components of ERPs. Theta and delta EROs underlying P3 are related to different cognitive functions: Theta is associated with memory processes and attention, and involves fronto-limbic or cortico-hippocampal interactions, and is taken as an index of frontal processing; reciprocal synchronization has been observed in the theta range between hippocampus and frontal and parietal regions in the brain during attentional tasks [66]. Delta is related to signal detection and decision-making, and is generated by cortico-cortical interactions and is prominent after the target stimuli.

In a visual oddball paradigm, alcoholics manifest significantly less evoked theta and delta ERO amplitudes while processing the target stimuli [121]; these findings are most significant anteriorly for theta, and posteriorly for delta. In order to determine whether these deficits in theta and delta oscillations antecede the development of alcoholism we examined adolescent high risk children of alcoholics compared to normal children, using the same paradigm [122]. The results showed that the adolescent offspring of alcoholics have reduced delta and theta band ERO amplitude (underlying P3) while processing the target stimuli compared to controls; differences were most prominent centroparietally for theta, and parietally for delta. The ERO measures have provided robust group differentiation and at times function as more sensitive measures than P3 in differentiating between high risk and low risk offspring. Thus, the results of these two studies demonstrate that decreased theta and delta EROs to target stimuli may antecede the development of alcoholism and represents a strong trait marker (Figure 29.3).)

We have reported significant linkage and association between the *CHRM2* gene on chromosome 7 and frontal theta oscillations to target stimuli in a visual oddball task; association analyses using both population-based tests (Measured Genotype) and pedigree-based tests (Quantitative Pedigree Disequilibrium Test, QPDT) indicate significant association of the frontal theta band ERO phenotype with several SNPs surrounding exon 4 of *CHRM2*. Further, an examination of the slower frequency parietal-occipital delta band EROs also revealed significant association with several SNPs [107, 108] (Figure 29.3).

These findings implicate a role of *CHRM2* in the generation and modulation of evoked oscillations. Theta and delta EROs depend on the level of acetylcholine (muscarinic activation). M2 receptors inhibit presynaptic release of acetycholine, leading to inhibition of irrelevant networks. Muscarinic receptors are especially concentrated in the forebrain and possibly serve to maintain the effective balance of relevant/

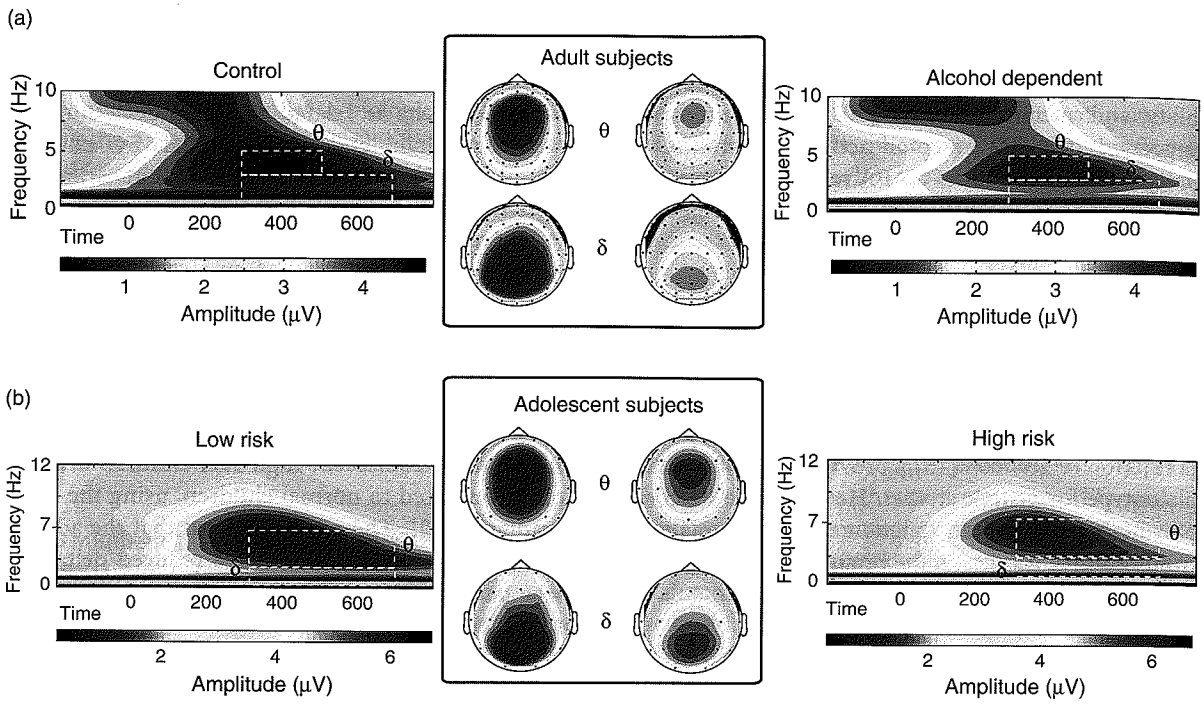

Figure 29.3 S-transform derived time-frequency representations of the average instantaneous amplitude that are z-scored for each frequency. Instantaneous amplitudes were averaged across individual trials of subjects so that nonphase locked or imprecise phase locked oscillatory energy is preserved. (a) Plots for the target condition at central (Cz) electrode in 120 alcoholic (right) and 120 control (left) subjects. Center panel – Topographic headplots of the time-frequency regions of interest (TFROIs) indicate that the theta band (4–5 Hz) power at 300–500 ms shows frontal maxima while the delta band (1–3 Hz) power at 350–700 ms has posterior maxima. Note that alcoholics have weaker responses in both theta and delta bands. (b) Plots for the target condition at frontal (Fz) electrode in visual oddball task in 87 high risk adolescent offspring of alcoholics from Collaborative Study on the Genetics of Alcoholism (COGA) families (right) and 57 matched low risk offspring of nonalcoholics from control families (left). Center panel – Topographic head plots for the TFROI that extends from 300 to 700 ms for each band revealing frontal maxima for theta band power and parietal maxima for delta band power. Note that high risk adolescents have weaker responses in both theta and delta bands to target stimuli, similar to the alcoholics. See plate section for color version.

irrelevant networks, hence, having a direct influence on P3 generation [123]. Genetic underpinnings of evoked oscillations are likely to stem from regulatory genes that control the neurochemical processes of the brain and, therefore influence neural function. The three major neurochemical substrates contributing to theta and delta rhythms and P3 involve strong GABAergic, cholinergic, and glutamatergic system interactions [123].

Moreover, the cholinergic muscarinic genes have a major role in memory and cognition [124, 125]. Several studies, including the COGA study, have found evidence that the *CHRM2* gene may be involved in intelligence [125–127]. In the COGA study, we found evidence of association with multiple SNPs across *CHRM2* and Performance IQ, as measured by the Wechsler Adult Intelligence Scale-Revised (WAIS-R). These results remain significant after taking into account alcohol dependence and depression diagnoses in the sample [126].

Evidence from the COGA project indicates that the *CHRM2* gene is not only associated with brain oscillations and cognition, but also clinical diagnosis. Significant linkage and association were reported for the *CHRM2* gene and a diagnosis of alcohol dependence and depression [128], comorbid alcohol and drug dependence (a more severe addiction profile) [129], as well as a spectrum of externalizing disorders [130]. Other groups have replicated these findings, reporting that the *CHRM2* gene predisposes to alcohol dependence, drug dependence and affective disorders [131], and major depression in women [132].

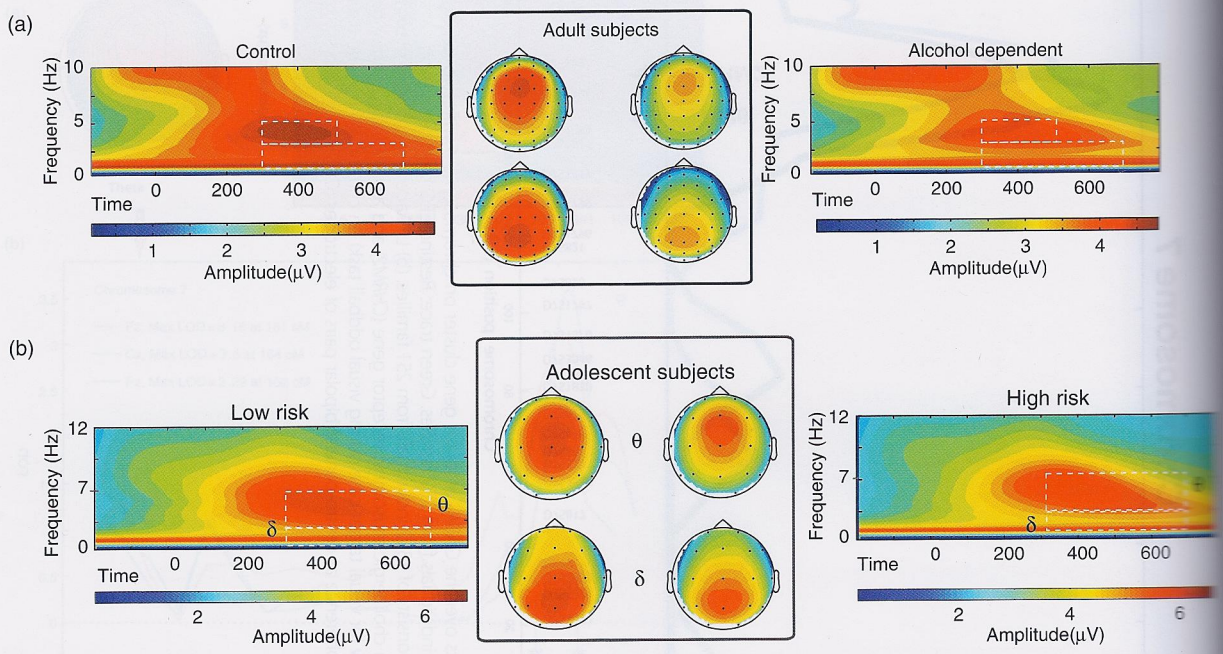

Figure 29.3 S-transform derived time-frequency representations of the average instantaneous amplitude that are z-scored for each frequency. Instantaneous amplitudes were averaged across individual trials of subjects so that nonphase locked or imprecise phase locked oscillatory energy is preserved. (a) Plots for the target condition at central (Cz) electrode in 120 alcoholic (right) and 120 control (left) subjects. Center panel – Topographic headplots of the time-frequency regions of interest (TFROIs) indicate that the theta band (4–5 Hz) power at 300–500 ms shows frontal maxima while the delta band (1–3 Hz) power at 350–700 ms has posterior maxima. Note that alcoholics have weaker responses in both theta and delta bands. (b) Plots for the target condition at frontal (Fz) electrode in visual oddball task in 87 high risk adolescent offspring of alcoholics from Collaborative Study on the Genetics of Alcoholism (COGA) families (right) and 57 matched low risk offspring of nonalcoholics from control families (left). Center panel – Topographic head plots for the TFROI that extends from 300 to 700 ms for each band revealing frontal maxima for theta band power and parietal maxima for delta band power. Note that high risk adolescents have weaker responses in both theta and delta bands to target stimuli, similar to the alcoholics.

Thus genes important for the expression of the endophenotype (brain oscillations) help in identification of genes that increase the susceptibility for risk of alcohol dependence and related disorders [68, 133].

Another likely candidate gene located under the same theta ERO linkage peak on chromosome 7 is a metabotropic glutamate receptor (*GRM8*) gene. The glutamatergic system is one of the major players modulating CNS electrophysiological networks, and in particular is also involved in theta oscillations and P3 [123]. Family-based association analyses of theta EROs revealed significant associations with several SNPs in the *GRM8* gene and theta EROs to target stimuli at frontal, central, and parietal regions [134]. An interesting finding is that several SNPs were also significantly associated with the diagnosis of alcohol dependence using ICD-10 diagnostic criteria.

A 3-T proton magnetic resonance spectroscopy (1H-MRS) study has suggested the involvement of glutamatergic neurotransmission in integrative frontal-hippocampal processing [135] and the sensation-seeking personality dimension [136]. The study demonstrated a robust relationship between glutamate levels in the hippocampus and frontal theta activity during auditory stimulus processing. Glutamatergic neurotransmission and its neuroadaptive changes have been proposed as important molecular determinants of craving and relapse [137, 138]. In particular, it is suggested that a hyperglutamatergic state mediates, at least in part, alcohol relapse behavior and maintenance of alcoholism [139]. Several studies have suggested the involvement of glutamate receptors – NMDA and metabotropic, in alcohol relapse [68, 140, 141]. Acamprosate, a drug used to prevent relapse in alcoholic patients [142], has been suggested to act through a suppression of a hyperglutamatergic state created by alcohol addiction [143, 144].

In light of the theta oscillations showing strong association to both *CHRM2* and *GRM8* genes, one could speculate a synergistic genetic mechanism underlying this electrophysiological phenotype, thus opening doors to future research in this direction. Interestingly, in studies on the rat hippocampus, authors have reported that a majority of interneurons strongly immunopositive for the muscarinic M2 or the mGlu1 receptors were the primary targets of mGluR8-containing terminals [145]. Rare neurons coexpressing calretinin and M2 were consistently targeted by mGluR8-positive boutons. The postsynaptic interneuron type-specific expression predicts a role in adjusting the activity of interneurons depending on the level of network activity.

Conclusions

Psychiatric disorders result from a complex interaction of changing genetic and environmental liabilities across development, possibly with greater genetic loading in individuals who have early onset of the disorders. The use of quantitative endophenotypes provides the power to more easily localize and characterize disease susceptibility genes than diagnostic categories. The measures of these endophenotypes reflect the genetic liability of the disorder among nonaffected relatives of affected individuals. Many of the same genes important for the expression of the endophenotypes help in identification of genes that increase the susceptibility for risk of the disease. Hence, as this chapter has illustrated, the utility of quantitative biological endophenotypes for the study of genetic risk of psychiatric disorders continues to be very promising.

Endophenotypes may have additional uses in psychiatry, including uses in diagnosis, classification, and the development of animal models. The lack of a biological basis for the classification of psychiatric disorders has led to limited success in research in the neurobiology and genetics of psychiatric disorders. Endophenotype-based analysis would be useful for establishing a biological underpinning for diagnosis and classification; a net outcome would be improved understanding of the neurobiology and genetics of psychopathology.

While the endophenotype approach is not a new idea, it is an approach whose time has come. Because of the rapidly evolving technology in the fields of molecular and statistical genetics, new methods are emerging that will facilitate the search for genes underlying complex traits in the near future. With the advent of SNP genotyping technology, genome-wide association studies using dense sets of SNPs across the genome, together with recent advances in novel statistical genetic techniques and computational power, have facilitated the identification of genes that are associated with heritable, genetically influenced quantitative traits that are risk factors involved in the etiology of various psychiatric disorders. Once genes are identified and understood, risk genotypes

and haplotypes can be studied in prospective studies of young individuals who have not yet developed the disease, and can lead to an improved understanding of how genes contribute to susceptibility, which in turn can lead to the design of well-targeted prevention initiatives.

References

1. Akerberg E. *Hereditas* 1986;**105**:1–5.
2. Gottesman II, et al. *Schizophrenia and Genetics: A Twin Study Vantage Point*. New York: Academic Press; 1972.
3. Lewis DA. *Am J Psychiatry* 2002;**159**:1467–1469.
4. Kandel ER. *Am J Psychiatry* 1998;**155**:457–469.
5. Tsuang MT. *Am J Psychiatry* 2000;**157**:489–491.
6. Gottesman, II, et al. *Am J Psychiatry* 2003;**160**:636–645.
7. John B, et al. *Science* 1966;**152**:711–721.
8. Munafo MR. *Genes Brain Behav* 2006;5 Suppl 1:3–8.
9. Skuse DH. *Br J Psychiatry* 2001;**178**:395–396.
10. Waldman ID. *Biol Psychiatry* 2005;**57**:1347–1356.
11. Walters JT, et al. *Mol Psychiatry* 2007;**12**:886–890.
12. Almasy L, et al. *Am J Med Genet* 2001;**105**:42–44.
13. Leboyer M, et al. *Trends Neurosci* 1998;**21**:102–105.
14. Almasy L, et al. *Am J Hum Genet* 1998;**62**:1198–1211.
15. Porjesz B, et al. *Clin Neurophysiol* 2005;**116**:993–1018.
16. Braff DL, et al. *Psychopharmacology (Berl)* 2001;**156**:234–258.
17. Braff DL, et al. *Arch Gen Psychiatry* 1990;**47**:181–188.
18. Geyer MA, et al. *Brain Res Bull* 1990;**25**:485–498.
19. Swerdlow NR, et al. *Biol Psychiatry* 1993;**33**:298–301.
20. Swerdlow NR, et al. *J Neurol Neurosurg Psychiatry* 1995;**58**:192–200.
21. Freedman R, et al. *Biol Psychiatry* 1983;**18**:537–551.
22. Siegel C, et al. *Arch Gen Psychiatry* 1984;**41**:607–612.
23. Clementz BA, et al. *Am J Psychiatry* 1998;**155**:1691–1694.
24. Myles-Worsley M. *Am J Psychiatry* 2002;**159**:2007–2012.
25. Myles-Worsley M, et al. *Biol Psychiatry* 1996;**39**:289–295.
26. Freedman R, et al. *Proc Natl Acad Sci U S A* 1997;**94**:587–592.
27. Freedman R, et al. *Am J Med Genet* 2001;**105**:20–22.
28. Leonard S, et al. *Arch Gen Psychiatry* 2002;**59**:1085–1096.
29. Munoz DP. *Prog Brain Res* 2002;**140**:89–96.
30. Hong LE, et al. *Arch Gen Psychiatry* 2006;**63**:259–264.
31. Kathmann N, et al. *Am J Psychiatry* 2003;**160**:696–702.
32. Lin PI, et al. *Schizophr Bull* 2008;**34**:791–797.
33. Arolt V, et al. *Am J Med Genet* 1996;**67**:564–579.
34. Matthysse S, et al. *Am J Med Genet B Neuropsychiatr Genet* 2004;**128B**:30–36.
35. Cannon TD, et al. *Am J Hum Genet* 2000;**67**:369–382.
36. Conklin HM, et al. *Am J Psychiatry* 2000;**157**:275–277.
37. Park S, et al. *Arch Gen Psychiatry* 1995;**52**:821–828.
38. Calkins ME, et al. *Schizophr Bull* 2007;**33**:33–48.
39. Gur RE, et al. *Schizophr Bull* 2007;**33**:49–68.
40. Greenwood TA, et al. *Arch Gen Psychiatry* 2007;**64**:1242–1250.
41. Gasperoni TL, et al. *Am J Med Genet B Neuropsychiatr Genet* 2003;**116B**:8–16.
42. Hovatta I, et al. *Am J Hum Genet* 1999;**65**:1114–1124.
43. Egan MF, et al. *Proc Natl Acad Sci U S A* 2001;**98**:6917–6922.
44. Sklar P. *Annu Rev Genomics Hum Genet* 2002;**3**:371–413.
45. Tunbridge EM, et al. *Biol Psychiatry* 2006;**60**:141–151.
46. Weinberger DR, et al. *Biol Psychiatry* 2001;**50**:825–844.
47. Doyle AE, et al. *J Child Psychol Psychiatry* 2005;**46**:774–803.
48. Doyle AE, et al. *Biol Psychiatry* 2005;**57**:1324–1335.
49. Rommelse NN. *Expert Rev Neurother* 2008;**8**:1425–1429.
50. Meyer-Lindenberg A, et al. *Mol Psychiatry* 2006;**11**:867–877, 797.
51. Callicott JH, et al. *Am J Psychiatry* 2003;**160**:709–719.
52. Mattay VS, et al. *Proc Natl Acad Sci U S A* 2003;**100**:6186–6191.
53. Meyer-Lindenberg A, et al. *Nat Neurosci* 2005;**8**:594–596.
54. van 't Ent D, et al. *Neuroimage* 2007;**35**:1004–1020.
55. Prasad KM, et al. *Schizophr Bull* 2008;**34**:774–790.
56. Burmeister M, et al. *Nat Rev Genet* 2008;**9**:527–540.
57. Kauwe JS, et al. *Ann Neurol* 2007;**61**:446–453.
58. Lindqvist D, et al. *Psychoneuroendocrinology* 2008;**33**:1061–1068.
59. Mann JJ, et al. *Biol Psychiatry* 2009;**65**:556–563.
60. Bartels M, et al. *Psychoneuroendocrinology* 2003;**28**:121–137.
61. Federenko IS, et al. *J Clin Endocrinol Metab* 2004;**89**:6244–6250.
62. Derijk RH, et al. *Eur J Pharmacol* 2008;**585**:492–501.

63. Gotlib IH, et al. *Biol Psychiatry* 2008;**63**:847–851.
64. Uhart M, et al. *Neuropsychopharmacology* 2006;**31**:2255–2263.
65. Kendler KS, et al. *Arch Gen Psychiatry* 2003;**60**:929–937.
66. von Stein A, et al. *Int J Psychophysiol* 2000;**38**:301–313.
67. van Beijsterveldt CE, et al. *Am J Hum Genet* 1996;**58**:562–573.
68. Bachteler D, et al. *Neuropsychopharmacology* 2005;**30**:1104–1110.
69. Bauer LO. *Neuropsychopharmacology* 2001;**25**:332–340.
70. Costa L, et al. *Drug Alcohol Depend* 1997;**46**:87–93.
71. Propping P, et al. *Hum Genet* 1981;**59**:51–59.
72. Rangaswamy M, et al. *Biol Psychiatry* 2002;**52**:831–842.
73. Winterer G, et al. *Psychiatry Res* 1998;**78**:101–113.
74. Finn PR, et al. *Alcohol Clin Exp Res* 1999;**23**:256–262.
75. Gabrielli WF, Jr, et al. *Psychophysiology* 1982;**19**:404–407.
76. Rangaswamy M, et al. *Neuroimage* 2004;**21**:329–339.
77. Bauer LO, et al. *J Stud Alcohol* 1993;**54**:577–589.
78. Begleiter H, et al. *Alcohol Clin Exp Res* 1999;**23**:1125–1135.
79. Porjesz B, et al. *Proc Natl Acad Sci U S A* 2002;**99**:3729–3733.
80. Edenberg HJ, et al. *Am J Hum Genet* 2004;**74**(4):705–714.
81. Agrawal A, et al. *Behav Genet* 2006;**36**(5):640–650.
82. Dick DM, et al. *J Stud Alcohol* 2006;**67**:185–194.
83. Dick DM, et al. *Behav Genet* 2006;**36**:577–590.
84. Covault J, et al. *Am J Med Genet B Neuropsychiatr Genet* 2004;**129**:104–109.
85. Drgon T, et al. *Am J Med Genet B Neuropsychiatr Genet* 2006;**141**:854–860.
86. Enoch MA, et al. *Am J Med Genet B Neuropsychiatr Genet* 2006;**141**:599–607.
87. Fehr C, et al. *Psych Genet* 2006;**16**:9–17.
88. Lappalainen J, et al. *Alcohol Clin Exp Res* 2005;**29**:493–498.
89. Soyka M, et al. *J Psychiatr Res* 2008;**42**:184–191.
90. Tobler I, et al. *Proc Natl Acad Sci U S A* 2001;**98**:6464–6469.
91. Haenschel C, et al. *Proc Natl Acad Sci U S A* 2000;**97**:7645–7650.
92. Faulkner HJ, et al. *Br J Pharmacology* 1999;**128**:1813–1825.
93. Kopell N, et al. *Proc Natl Acad Sci U S A* 2000;**97**:1867–1872.
94. Whittington MA, et al. *Int J Psychophysiol* 2000;**38**:315–336.
95. Abi-Dargham A, et al. *Am J Psychiatry* 1998;**155**:1550–1555.
96. Krystal JH, et al. *Arch Gen Psychiatry* 2006;**63**:957–968.
97. Lingford-Hughes AR, et al. *Br J Psychiatry* 1998;**173**:116–122.
98. Volkow ND, et al. *Alcohol Clin Exp Res* 1995;**19**:510–516.
99. Sarnthein J, et al. *J Neurosci* 2007;**27**:124–131.
100. Sarnthein J, et al. *Int J Psychophysiol* 2005;**57**:87–96.
101. Kaplan RF, et al. *J Stud Alcohol* 1985;**46**:122–127.
102. Michael A, et al. *Acta Psychiatr Scand* 1993;**87**:213–217.
103. Winterer G, et al. *Acta Psychiatr Scand* 2003;**108**:51–60.
104. Porjesz B, et al. *Scientific World J* 2007;**7**:131–141.
105. Winterer G, et al. *Am J Med Genet B Neuropsychiatr Genet* 2003;**117**:51–56.
106. Linas R. *Rebound Excitation as the Physiological Basis for Tremor: A Biophysical Study of the Oscillatory Properties of Mammalian Central Neurons In Vivo*. London: Macmillan; 1984.
107. Jones KA, et al. *Behav Genet* 2006;**36**:627–639.
108. Jones KA, et al. *Int J Psychophysiol* 2004;**53**:75–90.
109. Seeger T, et al. *J Neurosci* 2004;**24**:10117–10127.
110. Apergis-Schoute J, et al. *J Neurosci* 2007;**27**:4061–4071.
111. Begleiter H, et al. *Science* 1984;**225**:1493–1496.
112. Ardekani BA, et al. *Brain Res Cogn Brain Res* 2002;**14**:347–356.
113. Halgren E, et al. *Science* 1980;**210**:803–805.
114. Kiehl KA, et al. *Schizophr Res* 2001;**48**:159–171.
115. Menon V, et al. *Neuroreport* 1997;**8**:3029–3037.
116. Basar-Eroglu C, et al. *Int J Psychophysiol* 1992;**13**:161–179.
117. Basar E, et al. *IEEE Eng Med Biol Mag* 1999;**18**:56–66.
118. Karakas S, et al. *Neuroscience Lett* 2000;**285**:45–48.
119. Karakas S, et al. *Clin Neurophysiol* 2000;**111**:1719–1732.
120. Yordanova J, et al. *Neuroreport* 1996;**8**:277–280.
121. Jones KA, et al. *Clin Neurophysiol* 2006;**117**:2128–2143.
122. Rangaswamy M, et al. *Int J Psychophysiol* 2007;**63**:3–15.
123. Frodl-Bauch T, et al. *Neuropsychobiology* 1999;**40**:86–94.
124. Calabresi P, et al. *Eur J Neuroscience* 1998;**10**:3020–3023.
125. Comings DE, et al. *Mol Psychiatry* 2003;**8**:10–11.
126. Dick DM, et al. *Behav Genet* 2007;**37**:265–272.
127. Gosso FM, et al. *BMC Med Genet* 2007;**8**:66.
128. Wang JC, et al. *Hum Mol Genet* 2004;**13**:1903–1911.

129. Dick DM, et al. *Addiction* 2007;**102**:1131–1139.

130. Dick DM, et al. *Arch Gen Psychiatry* 2008; **65**:310–318.

131. Luo X, et al. *Hum Mol Genet* 2005;**14**:2421–2434.

132. Comings DE, et al. *Am J Med Genet* 2002; **114**:527–529.

133. Begleiter H, et al. *Int J Psychophysiol* 2006;**60**:162–171.

134. Chen AC, et al. *Am J Med Genet B Neuropsychiatr Genet* 2009;**150B**: 359–368.

135. Gallinat J, et al. *Psychopharmacology* 2006;**187**:103–111.

136. Gallinat J, et al. *Neuroimage* 2007;**34**:671–678.

137. Cornish JL, et al. *J Neurosci* 2000;**20**:RC89.

138. Tzschentke TM, et al. *Crit Rev Neurobiol* 2000; **14**:131–142.

139. Tsai G, et al. *Annu Rev Med* 1998;**49**:173–184.

140. Holter SM, et al. *Pharmacol Biochem Behav* 2000; **66**:143–151.

141. Krystal JH, et al. *Pharmacol Ther* 2003;**99**:79–94.

142. Mann K, et al. *Alcohol, Clin Experiment Res* 2004; **28**:51–63.

143. Dahchour A, et al. *Prog Neurobiol* 2000;**60**:343–362.

144. Spanagel R. *Addiction* 2005;**100**:1813–1822.

145. Ferraguti F, et al. *J Neurosci* 2005;**25**:10520–10536.