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Genomics

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1 EXECUTIVE SUMMARY

Inherited genetic make-up contributes importantly to sensitivity to drugs and their effects, and to behavioural traits that may predispose to compulsive drug taking. In addition, the effects of drug taking, along with other environmental influences, act to influence future behaviour by changing gene expression, both during development and in adulthood. The genomics of addiction encompasses both inherited determinants of addictive behaviour and the effects of drug taking on gene expression. Additionally, genetic influences on the utility of addiction treatments are potentially, and controversially, of interest.

Evidence that genetic make-up influences drug addictions is provided by natural experiments in the form of family, twin and adoption studies. Such approaches provide convincing evidence that genes are implicated in addictive behaviour. An estimate of the relative genetic contribution is also provided by twin studies. For example, in alcohol dependence, typical figures for the role of genetic factors are approximately 50% in males and 25% in females. In smoking, figures for the genetic contribution to smoking initiation and maintenance are approximately 60%. Heritability estimates for the abuse of, or dependence on, various substances including cannabis, opiates, stimulants, sedatives and psychedelics are around 45%.

These figures demonstrate the importance of genetic factors, while also recognising the contribution of environmental and other influences on addiction development. Two main approaches have been adopted to identify the specific genes involved, namely linkage and association. Linkage, using pedigrees, has suggested that several chromosomal regions may be implicated in addiction, but as yet it has not identified specific genes. Association studies have provided strong evidence for a role for the alcohol-metabolising enzymes in alcohol dependence, but the evidence is less robust for the other multiple genes implicated in addiction. Complementary studies in animals selectively bred for addiction-related traits confirm the importance of inheritance in addiction, and have identified a number of chromosome regions and specific genes that are associated with the selected trait. It is important to appreciate that in both human and animal studies, as with other complex behavioural traits, many genes are associated with addiction, and the contribution made by any particular gene may be very small.

The human genome project has identified some 25,000–30,000 'genes' as making up the human genome. Most of these genes are susceptible to alterations called single nucleotide polymorphisms (SNPs). Although many or most SNPs will have no functional consequences, others will contribute to the addiction-related behavioural phenotype, adding another layer of complexity to understanding.

Animal studies offer the advantage over human studies that particular genes can be deleted, inserted or modified and the consequences studied. In contrast, gene expression analysis allows the influence of drug taking on gene function to be investigated. Human and animal studies reveal that drug taking is associated with the change in expression of dozens or hundreds of genes. The pattern of change may vary with the tissue studied, or, in the case of the

brain, between brain regions. It will take several years before we understand the complexity of drug influences on gene expression, and the implications for neuronal and behavioural plasticity that may contribute to future addictive behaviour.

1.1 Future research

Further human research in this area requires the collection of large-scale, well-characterised samples exploiting international, multidisciplinary collaborations and adopting universally agreed protocols. In addition, longitudinal studies will aid the identification of gene–environment interactions that occur during the dynamic process of development, and that at present may be obscuring the genetic effects. Furthermore, technological advances will permit the systematic examination of the human genome for any role in addiction.

1.2 Future impact

To date, genetic approaches have failed to deliver on their early promise and have not yielded any major advance in the management of addiction. However, the identification of the genetic underpinning of addiction, in its developmental interplay with the environment, will have a profound effect on the field. This will have important implications for the management of addiction from initiation, through the development of tolerance, dependence and physical complications, to treatment response, relapse and ultimately recovery. Due to the large number of genes of small effect that are likely to be involved in addiction, it is anticipated that genetic screening of the population for vulnerability will not be realistic. The elucidation of the genetic, environmental and developmental factors will strongly influence public attitudes, political strategies and health policies.

2 GENES IN ACTION

2.1 Genes and development

Variation in people's response to physical, nutritional, drug and psychosocial 'exposures' is determined by inherited differences, different developmental experience or both. Genetics (the study of heredity and how traits are passed on through generations) and genomics (the study of an organism's genetic make-up) will inform our understanding of both these influences and of their interaction. This is true of vulnerability to addiction, of the body's neurochemical response to drug use, and of the consequences of addictions in terms of organ damage by specific agents and the response to either treatment by drugs or psychotherapy. The complexity of the factors that contribute to drug addiction implies that genes play many different roles.

Our 25,000 or so genes are specific sections of DNA that can be 'read' or transcribed, dictating the formation of corresponding functional RNA molecules, which either act directly or are translated to produce corresponding amino-acid or polypeptide chains that go to make up proteins

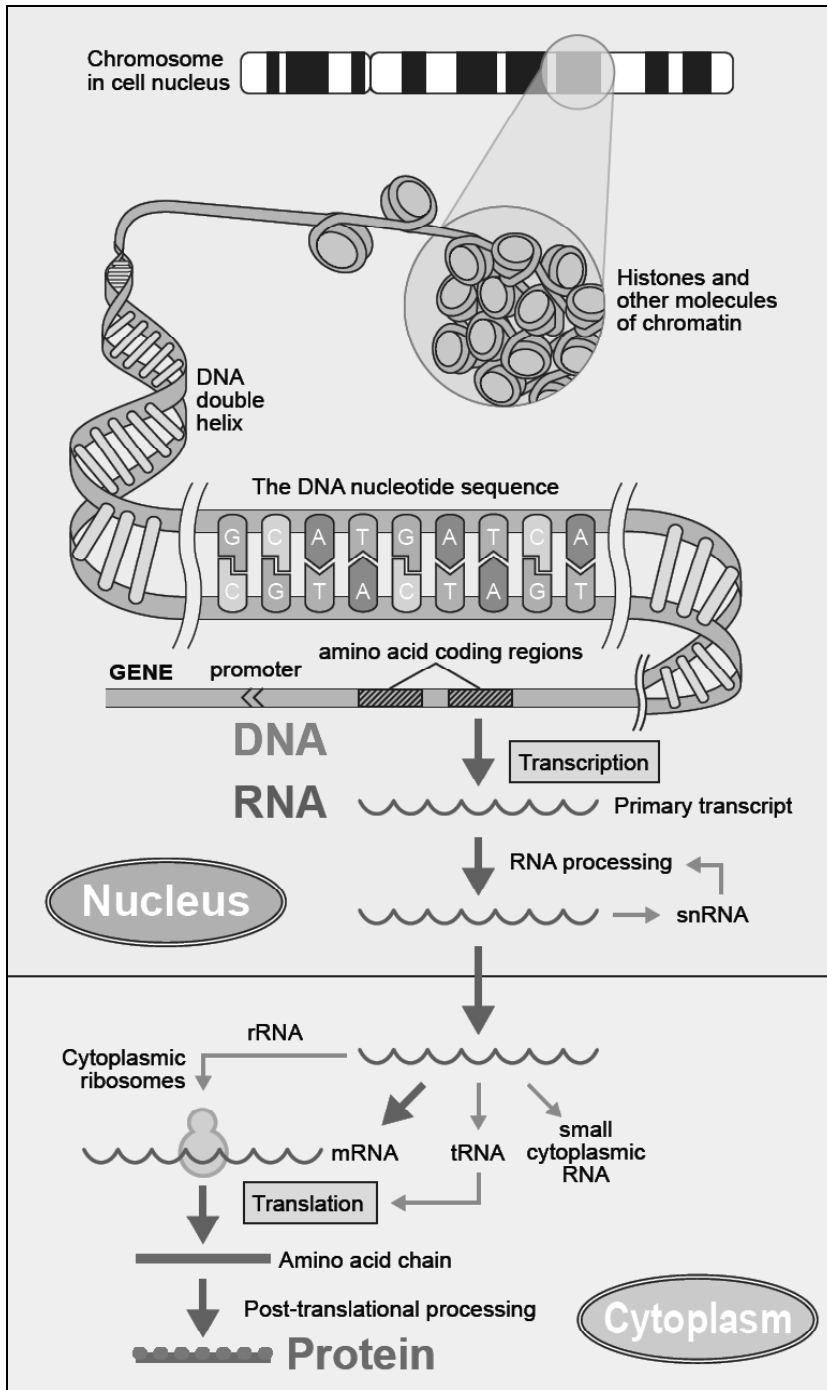
(Figure 1). It is the nature and abundance of specific proteins that shape the structural and functional differences between cells from the starting point of development, the fertilised egg, onwards. However, during the process of translation from the DNA sequence to the amino-acid chain encoded by the gene, a number of editing steps may occur. So, although the human genome may contain only 25,000 genes, the body is able to use the information encoded to produce hundreds of thousands of different proteins. Furthermore, although all cells in the body (with a few exceptions, such as red blood cells) possess the entire complement of DNA, only a proportion of the genes are used to determine which proteins are synthesised in any particular cell, at any particular stage of development. Control of the expression of genes is as important as the genetic complement in determining the final product of the genome (i.e. the individual), and such control is itself subject to both genetic and environmental influences.

The transfer of biological information from one generation to the next is mediated in three main ways, the arrival of a fully functional cell in the form of the ovum, the DNA sequence of half the mother's and half the father's chromosomes, and the transplacental flow of molecules from the mother to the developing foetus. It is DNA-sequence variation and maternal-foetal interactions that determine most of the differences between newborns. However, the portrayal of gene action in Figure 1 only shows the flow of information in one direction. The cells are constantly interacting with the world around them. Cells sense the environment (including physical factors, nutritional status, exposure to drugs and chemicals, infection, and psychosocial experiences) and respond by sending internal signals to change their gene expression and therefore the way the cell develops or functions.

There is a tendency to regard the human 'genome' as being merely the complete DNA nucleotide sequence of its chromosomes and, if one is being thorough, adding in the sequence of the small circular chromosomes in the mitochondria as well. But within cell nuclei, functional DNA is incorporated into chromatin, which consists of histone and non-histone proteins bound to genomic DNA. The accessibility of DNA sequences for transcription regulatory factors (molecules or complexes of molecules that bind to DNA and determine gene expression levels) depends on the chromatin state. This in turn can mean that developmental experience can be captured as changes in chromatin state leading to more or less permanent changes in gene expression. The constant two-way flow of information modulating cell function through changes in gene expression is central to the role of genetics and genomic research in drug addiction and its treatment. This has to be borne in mind throughout the sections of this review that focus on inherited differences between people. In the final analysis, we are usually dealing with differences in response, even if the developmental outcome is a more or less fixed change in gene expression that then contributes to what used to be called a person's 'constitution'.

Figure 1: Basic structure and function of the genome

A schematic summary of the structure of the genome from chromosome (visible under the light microscope) down to the DNA nucleotide sequence, and the function of the genome through transcription of the DNA code of the gene into RNAs with mRNA (messenger RNA) being translated into protein. As emphasised in the text, this figure portrays the action in one direction only, namely gene expression. In response to signals from the internal and external environment, gene expression is triggered by molecular complexes binding to the promoter region of the gene to either activate the gene by promoting transcription, or silence the gene by locking it into tightly packed chromatin.



2.2 Relating genes to addiction

We all know what 'addiction' is meant to mean, but when it is discussed, different individuals will emphasise different aspects. Different mechanisms may underlie the propensity to abuse drugs and the propensity to become dependent upon them. Liability to addiction will reflect a mixture of psychological, neurobiological and other biological traits. Thus, certain addictions may be related to a trait of risk taking. But a risk-taking trait will not always reveal itself as a tendency to take drugs. It may appear as promiscuity, free-fall parachute jumping, or investing on the stock exchange.

At a neurobiological level, most drugs of abuse are known to affect, directly or indirectly, neurotransmission in pathways using the neurotransmitter dopamine. These pathways have been long implicated in mediating the ability of rewards (including drugs) to influence behaviour. There is emerging evidence that particular alleles of genes encoding the dopamine D2 receptor (DRD2) may be associated with a reduction in the efficiency of this system and thus a reduced ability to experience reward. Individuals with such genes may be predisposed to take drugs to restore the activity of these systems. However, even if such a relationship were proven, it is clear that the DRD2 gene is not simply a gene for addiction, but is involved in much wider brain functions. Other genes may encode proteins that are essential for the breakdown of drugs. Variations in such genes will affect the rate of metabolism of the drug, which may make it more or less addictive. This shows that understanding the contribution of genomics to drug addiction will need the integration of evidence from many fields. Identifying variations in genetic constitution between groups of addicts (even if they are very clearly defined) and non-addicts will provide only a glimmer of the knowledge needed to understand the particular role of any gene.

2.3 Inherited variation

2.3.1 *What contribution do genetic variants make to behavioural differences?*

A key question in human biomedical research is the contribution of inherited variation to differences between people, including differences in vulnerability to or recovery from addiction, and differences in susceptibility to damage from specific agents or in response to treatment. The primary source of inherited variation is differences in the DNA sequence that alter the gene product or its expression. There is also emerging evidence that certain chromatin states at particular genes may be transmitted from parent to offspring (Rakyan *et al.* 2003) but it is far too early to know whether this will turn out to be an important determinant of the offspring's health or just a curiosity.

Discovering which DNA-sequence variant contributes to what variation in response is important for two reasons. If the genetic effect is substantial, testing for the DNA-sequence variant might allow people to be forewarned about their vulnerability or help them select 'tailor-made' therapy. Equally important, a causal link between a gene variant and an aspect of variation in response to psychoactive agents reveals something about the biological mechanisms involved, even if the link is mediated in a complex way. This is true even if the effect is small. So, discovering a specific genetic modulation of

a response and showing that it contributes to the differences between people is a powerful, independent way into the physiological and biochemical pathways mediating that difference. Such insights can lead to experimental verification in animals and a more logical development of therapy.

Most behavioural traits are influenced by a number of genes, each of which may exist in different forms called alleles. Variations in a behavioural trait are likely to be the consequence of differences in the contributions of different alleles for different genes. By cross-breeding strains with and without a behavioural trait of interest (such as the tendency to consume large quantities of alcohol), and tracking the trait across generations, it is possible, using DNA-sequence variants as chromosomal markers, to determine the approximate chromosomal location of genes contributing to it. A chromosomal locus that is important in determining a continuous character is called a quantitative trait locus (QTL). Chromosomal mapping of a QTL is only the first step. One is then faced with identifying which of the genes in that region are relevant. This task has been made much easier by the various 'genome projects' that have sequenced the entire genomes of man and several experimental species.

2.3.2 *Human genetic variation*

There is currently a worldwide effort to define the common and less common DNA sequence differences between people, both within and between human populations. The simplest and cheapest DNA variants to analyse are single nucleotide polymorphisms (SNPs). It has been estimated that there are 10–15 million SNPs whose more common allele occurs in at least 1% of genomes (Botstein and Risch 2003; Kruglyak and Nickerson 2001). Much of the international effort to date has been focused on defining these SNPs by sequencing suitable panels of samples (for example, the International HapMap Project (IHMP) 2003) and the work of Perlegen Inc (<http://www.perlegen.com>). By 2005, large-scale sequencing studies have trebled the number of markers that are now available, to a total of 8 million SNPs, an average of one for every 360 nucleotide base pairs in the genome, and 1 million deletion/insertion polymorphisms (DIPs) (dbSNP build 121 at <http://www.ncbi.nlm.nih.gov/SNP> and Mullikin, personal communication).

Despite these rich resources, our current knowledge of common SNPs in the genome is incomplete and there is much we don't know about other types of genomic variation. There can be chromosomal rearrangements, large-scale gene copy-number variation (Carter 2004), variation in the length of repeat sequences, including the length of the chromosome end 'caps' (telomeres) (Blackburn 1991) and insertion/deletion variants beyond single nucleotides. Any of the stable, common DNA polymorphisms can be used as 'markers' of a particular locus on the chromosome and can be used for co-inheritance studies within families with a particular disorder to map a susceptibility locus.

2.3.3 *Which variants are important in trait and disease-risk differences*

We are still largely ignorant of what classes of genetic variants are likely to be most relevant to human differences. Will common variants with minor allele

frequencies of more than 10% be the major players, or are we talking about a combination of multiple rare variants in a gene? Theoretical arguments have been made for both (Lander 1996, Pritchard 2001). Clearly, a variant that changes the coding for an amino acid is a good candidate and by 2005 the sequences of tens of thousands were publicly available (dbSNP release 121; <http://www.ncbi.nlm.nih.gov/SNP/>). At least 10,000 of these had been validated. However, variation in the regulatory regions around genes may prove to be more important for common disease (Rockman and Wray 2002). It is also noteworthy that while transcription-factor binding-site mutations are relatively rare in the monogenic disorders, variants deleting these sites in the normal population seem to be common (Wjst 2004). It is possible that such deletions will contribute to susceptibility to common diseases.

In terms of genetic influences in common traits and disorders, we are likely to be dealing with the combinatorial effects of variants in the coding region, promoter and other non-coding *cis*-regulatory DNA sequences, and sequences for functional non-protein-coding RNAs. Furthermore, the transient changes in the level of the activity of a gene in response to some experience is mediated by DNA-binding proteins called transcription factors, which are themselves gene products encoded by genes elsewhere in the genome. An inherited difference in that response may be due to DNA variants in the transcription factors rather than the target gene in question. It is one thing to note inherited variation in a gene's expression, quite another to discover the DNA variant or variants that underlie it.

2.4 Using genetics to dissect the evolved developmental/regulatory response

2.4.1 Top-down and bottom-up approaches

With addiction and other effects of psychoactive agents, like most areas of biomedical research, the ultimate aim must be to understand the underlying evolved regulatory responses. By evolutionary necessity these will be coherent, if complex, sets of gene–gene and gene–environment interactions and therefore their discovery is a tractable task (Pembrey 2004, Gottesman and Hanson 2005). This fact is the starting point for a bottom-up approach to discovery that also allows a very productive shuttling back and forth between laboratory animal experiments and human studies, both observational and interventional, each generating hypotheses to be tested in the other. It is already clear that there are commonalities in the genes underlying addictive behaviours in humans and in animal models.

Human studies most frequently investigate the genetic make-up of populations with a known susceptibility to abuse drugs. Animal studies adopt a variation of this top-down method but also allow bottom-up approaches. Top-down methodologies take a particular characteristic of the animal, such as a trait to consume alcohol, and seek to identify the processes, including genetic influences, that contribute to it. In this respect they are analogous to most human genetic studies. They often require the discovery of the genetic constitution of animal strains selectively bred for a particular behavioural trait, but the same approach can be used to study the consequences of taking a drug, or of withdrawal from a drug, on gene expression. A strength of this

approach is that the experimenter deliberately eschews hypotheses regarding the relationship of particular genes to addictive traits. With 25,000 genes potentially contributing to addictive behaviour, which itself has many different aspects, testing the importance of each individual gene in every possible way that might contribute to addiction is an impossible task. Simply studying which gene variations are associated with the addictive trait is more tractable, even though it tells us little about the particular function of any genes that are identified.

In contrast, bottom-up approaches start off with a question about the function of a particular gene, and how modifying it alters an animal's behaviour. The manipulation of an individual's genes is ethically unacceptable (as well as technically intractable) for human studies. Temporary experimental manipulation of gene function with drugs may offer an acceptable approach in humans in some circumstances. Such bottom-up approaches can address hypotheses regarding the importance of individual genes.

There are several methods available for such manipulations. Genes of interest may be deleted in mice using molecular biological techniques called gene knockouts, or extra copies may be inserted into the mouse genome, a technique called transgenics. These techniques have provided new information that is not available using more traditional techniques. A major surprise was the discovery that the dopamine transporter at which cocaine was assumed to act is not necessary for the rewarding properties of cocaine (Rocha *et al.* 1998; Sora *et al.* 1998), forcing behavioural neuroscientists to reconsider the mechanisms by which cocaine achieves its effects. The discovery that deleting the gene-encoding receptors for the neuropeptide Substance P abolishes the rewarding, but not the analgesic, properties of morphine (Ripley *et al.* 2002; Murtra *et al.* 2000; De Felipe *et al.* 1998) suggests the possibility of producing powerful pain killers without dependence or abuse.

However, it is increasingly recognised that such genetic manipulations may have influences on the broader development of the organism. The resulting test animal may differ in its response to drugs in several ways, some perhaps independent of the role of the targeted gene in the drug response of normal adult animals (Stephens *et al.* 2002). Knockouts offer an important refinement to this technique. Gene deletion in the germ line will influence development, and perhaps allow related genes to take over parts of the role of the deleted gene, although if the gene is knocked out late in life, it will not affect development, nor are compensatory mechanisms as likely to be activated. Furthermore, the manipulation of genes encoding for proteins that are used in multiple systems may have a range of behavioural effects. Disentangling them will be easier if the genetic manipulation is limited to a single tissue. Conditional knockouts have not yet been used widely in addiction research but are likely to become more frequent in the future.

In keeping with our knowledge that behavioural traits are unlikely to be attributable to single genes, the consequences of manipulating single genes using knockout techniques may differ depending on the genetic background of the mouse strain in which the knockout is made. Thus, deletion of the DAT

gene has rather different consequences for cocaine reward depending upon the mouse strain in which the knockout is created (Morice *et al.* 2004).

A novel way to increase gene expression in a particular area of the brain, and thus to test that gene's involvement in a particular aspect of addictive behaviour, is to incorporate the gene into the genetic material of a virus modified to make it harmless to humans, and to inject the virus into the area of interest in the brain. Once the virus penetrates a nearby nerve cell, it will produce products of the inserted gene, which the nerve cell will then incorporate in its own machinery, leading to an increase in the levels of that particular protein. This exciting technique has already been used to suggest an association of the DRD2 with reduced alcohol consumption (Thanos *et al.* 2001), and of the *Gria1* gene encoding GluR1 subunits of glutamate receptors in sensitisation to cocaine (Carlezon *et al.* 2002). Such gene-transfer technology may be used in the near future to re-insert genes in specific brain areas of mice from which the native genes have been deleted in knockout experiments. If this reinstates a disrupted behaviour, then the causal relationship between the gene and behaviour is firmly established.

In point of fact, the top-down and bottom-up approaches are complementary, so that, for instance, genes identified by microarray studies of chromosomal loci identified by the QTL approach can be tested directly by targeted gene-knockout technologies. It is unlikely, however, that genes identified in this way can be described as genes for addiction. Genes encode proteins, and those proteins are likely to be involved in many different essential bodily processes with very indirect relationships to the processes underlying addiction.

2.4.2 Towards systematic bottom-up approaches in humans

Individuality can be found at each '-omic' level from genome, through transcriptome, proteome and metabolome (Scriver 2004, Gottesman and Gould 2003). One general principle of the bottom-up approach can be applied in humans. The bottom-up approach asks what a particular gene or genetic variant does. This question is independent of any preconceived idea of disease category or phenotypic classification, although data have to be collected on the full range of phenotypic 'outcomes' to answer the question. Such planned approaches, exploiting phenotype-rich cohorts, have been dubbed the 'Human Phenome Project' (Freimer and Sabatti 2003) or Phenome Scans (Pembrey 2004). If our current phenotypic labels or measures map poorly onto the evolved biological mechanisms that underlie human response to the availability and use of psychoactive agents, then research designs such as case-control approaches, which start with such labels, may not be optimal for discovering specific genetic influences. Recognising this possibility has led to the incorporation of intermediate phenotypes into case-control types of research on the grounds that these may be closer to sites of gene action (Gottesman and Gould 2003).

It is also important to recognise that most genes and their genetic variants are likely to have multiple effects that cut across the way we currently carve up disorders into medical specialties based largely on organ systems.

2.4.3 *The relevance of the timing of developmental experience*

An important aspect of how people are moulded by developmental experience is whether particular susceptibility periods exist during development and how reversible the effects of certain exposures during these times may be. On first principles one might expect exposure during foetal life and early childhood to influence development most directly and permanently, a view reflected in our attempt to protect the foetus and infant from drug exposure. The effects of foetal exposure to maternal alcohol consumption (Mukherjee *et al.* 2005) and cigarette smoking (DiFranza *et al.* 2004) are obvious examples. In both instances, these effects are likely to be modified by genetic variants in the mother and the foetus. There is also experimental rodent evidence that adolescence may represent a window of vulnerability to the consequences of psychoactive drugs, including addictive behaviour (Adriani and Laviola 2004). From the genetic perspective, the question is how exposure at these critical times brings about the long-term changes in gene expression that underlie observed changes in brain function and molecular structure. While longitudinal observational studies in humans can look at the behavioural and certain molecular consequences of foetal exposure through maternal use in pregnancy or direct use later in childhood, animal experiments can attempt to define the mechanistic link with changed gene expression.

3 GENETIC ANALYSIS

3.1 Principles, problems and potential solutions

3.1.1 *Family and twins studies*

The traditional starting point for the study of genetic influences is to carry out family studies and twin studies to estimate the 'heritability' of a condition, crudely speaking an attempt at estimating how genetic it is. Just the use of this term is controversial (Guo 2000) because it is widely misunderstood. Its magnitude is not a reliable measure of the inherited component, especially of complex traits. Even RA Fisher (1951), a founder of heritability studies, called it 'an unfortunate shortcut'. The principle is to compare the occurrence of the condition of interest between two relatives, or unrelated people, in relation to the proportion of genes and genetic variants they would be expected to share by inheritance from a common embryo, parents or ancestors. This is 100% for monozygotic (identical) twins and 50% for dizygotic (non-identical) twins. If a condition has a significant genetic component, provided the environmental factors making them similar or different are approximately the same, the greater concordance in monozygotic twins should be due to their greater genetic similarity. Heritability is essentially a measure of familial aggregation which can be caused by genetic effects alone, by familial aggregation of environmental factors alone, or by a combination of genetic and environmental factors and their interactions. Moreover, the magnitude of the measure is determined not only by the strength of genetic and environmental effects and their interactions, but also by gene and environmental exposure frequencies, so it is a statement about a particular population under a particular set of environmental circumstances (Guo 2000). Thus, while results

from family studies can be consistent with genetic influence, they do not represent proof due to confounding from shared environment, and further support has been derived from adoption studies. These natural experiments provide the opportunity to study individuals reared by unrelated parents.

Both family and twin studies depend on defining the disorder of interest, which immediately raises the difficulty of how to classify a relative or twin who has an overlapping phenotype or a different but similar condition, such as addiction to a different agent. However, this difficulty can sometimes be exploited to raise testable hypotheses on underlying traits linking outcomes to gene effects. Accepting the provisos flagged up above about heritability, multivariate twin modelling has been used to dissect the structure of genetic and environmental risk factors for common psychiatric and substance-use disorders, where co-morbidity is common (Kendler *et al.* 2003). Heritability estimates will always have their limitations and will no doubt continue to produce newspaper headlines such as 'the infidelity gene' (BioNews 2004) or similar misconceptions. Traditionally, a heritability estimate is often the starting point of a family and twin study that goes on to include genetic linkage and genetic association studies, although its magnitude is a poor guide to the chances of success of such studies.

Human studies to elucidate the genetic contribution to complex behaviours and psychiatric disorders have mainly adopted one of these two strategies (see Sham and McGuffin 2002). A linkage or family study 'maps' a putative gene variant that is causing or contributing to a disorder in a particular chromosomal region. An association study, on the other hand, identifies a gene variant or variants that occur more often in people with the disorder compared to those without the disorder.

3.1.2 Linkage

Thanks to the human genome project and other research, we know the location of all the identified SNPs and other genetic variants along the chromosomes. Analysing a suitable set of such variants ('markers') in families allows one to follow the transmission of any chromosomal region despite the recombinations that occur during egg and sperm formation. Thus, in linkage approaches, the inheritance of a trait or disorder is tracked through pedigrees in an attempt to identify a co-inheritance between a genetic marker and the condition. If linkage is identified, this would implicate a gene, in the broad region around the marker, in the development of the disorder. Linkage studies are ideal for the systematic identification of genes of major effect, but the regions they identify are large. In addition, many of the approaches require the estimation of several unknown parameters, including penetrance and phenocopy rate, which are essentially best guesses. As such they are less useful for identifying the relatively modest individual genetic contributions that are anticipated in most psychiatric disorders and complex behaviours, such as alcohol dependence.

Genetic linkage studies of simply-inherited, monogenic disorders with a clear-cut phenotype are now straightforward provided sufficient, large enough families can be recruited to the study. The genotyping challenges of genetic linkage studies are essentially solved, whether one is dealing with Mendelian

disorders or more complex familial disorders where susceptibility loci are likely to exist. However, problems remain with complex traits, the main ones being the expected small effect of individual causal variants and the fact that the chromosomal region identified can be quite large. While there have been notable examples of success in linkage studies of common disease phenotypes (e.g. Calpain-10 and T2D (Horikawa *et al.* 2000), *ALOX5AP* and CHD (Helgadottir *et al.* 2004), *DPP10* and asthma (Van Eerdewegh *et al.* 2002)), there are also many examples of studies that have not yielded robust outcomes (Altmuller *et al.* 2001). In the absence of prohibitively large samples, the linkage approach is not well suited for complex traits with variants that contribute only modestly to the overall disease risk (Risch 2000). Attention has turned to the association approach as a preferred genetic strategy for identifying novel aetiological loci via genome-wide scanning (Botstein and Risch 2003; Risch 2000).

3.1.3 Association

Genetic association studies usually attempt to detect a difference in the distribution of a genetic variant between a sample of unrelated individuals with a particular phenotype, such as a diagnosis of alcohol dependence, and a matched control sample. As such they have the advantage of not requiring the estimation of unknown parameters. Association studies can also be used to detect genetic associations with a continuous variable or trait. In addition, in contrast to linkage studies, association is capable of detecting gene variants of relatively small effect, such as those that are more likely in alcohol dependence, although many thousands of markers would be required for a systematic approach that could screen the entire human genome. As a consequence, association studies have until now usually adopted the candidate-gene approach, examining DNA variants within genes on the basis of an *a priori* suspicion of a role in, say, alcohol dependence.

Two strategies have been adopted for association studies – direct and indirect. The former relies on identifying functional variants in the genes such that any association identified is very likely to be due to the DNA change itself and therefore replicable in other comparable studies. The second approach relies on linkage disequilibrium (essentially a co-inheritance of genetic variants close together on the chromosome) between markers such as SNPs, presumed to be non-functional in the condition, and the putative susceptibility or causal variant. Both approaches can be employed simultaneously.

3.1.3.1 Indirect association studies

The great hope has been that linkage disequilibrium (LD) can be exploited in genome-wide association studies to capture most of the human disease associations with relatively little genotyping. The \$100 million International HapMap Project was predicated on just such a strategy, but the jury is still out on how much of a short cut this will prove to be in the end. There is, for example, the problem of replication of the initial discovery in a population with different allele frequencies or haplotype structure (Neale and Sham 2004). Linkage disequilibrium is caused by the co-inheritance of genetic variants close together on the chromosome. In general, the closer alleles are on a

chromosome, the less likely they are to be separated during egg and sperm formation. Thus short arrays of alleles on a chromosome, so-called haplotypes, are transmitted intact and become established in populations until they are eventually disrupted by recombination or by a new genetic variant arising. It happens that meiotic recombination tends to occur at specific recombination 'hot spots' along the chromosomes, generating relatively stable 'haplotype blocs' in between. Thus, it was hoped that if a few easily genotyped SNPs could be found that were a reliable signature or 'tag' for each haplotype bloc, these could be used to detect disease associations with any of the functional causal genetic variants within the haplotype bloc. Just the associated haplotype blocs could then be genotyped extensively to find the causal variants. If only haplotype-based association screening was that simple! Haplotypes vary between populations, and haplotype blocs are a (rather arbitrary) matter of degree of LD, the blocs often breaking up into different haplotypes as a greater density of SNPs are typed. Clearly more cost-effective genotyping on more population samples will lead to better and better descriptions of the LD structure of human genomes worldwide. This is fine for 'out of Africa'-style population research into human evolution, diversity and recent migrations, but how relevant it is to disease or trait association studies is still unknown. Any predictions are at the mercy of our ignorance of which classes of genetic variants are most likely to underlie genetic susceptibility or resistance to common disorders.

3.1.3.2 Some current issues in association studies

Statistical issues in multiple testing. Association approaches are also very prone to type-1 errors or false positives, hence the importance of replication studies. For example, if a single test identifies an 'association' with a P value of 0.05, we recognise that this result could have occurred by chance once every 20 times. The question then arises of how we know that a positive result is a genuine finding. Buckland (2001) explores this in the context of alcoholism. In his analysis, he assumes that all genes have an equal chance of association, that there are 20 genes truly associated with alcohol dependence, 80,000 genes are in the human genome (an overestimate according to latest figures) and that the role of each gene can be tested using a single genetic polymorphism. Thus, examining 80,000 genes would result in a true association every 4,000 polymorphisms studied. Furthermore, any finding with a P value of 0.05 would have a 20/4,000 chance of being true, 0.5%. Conversely that means that it has a 99.5% likelihood of arising by chance. If there are a mere 30,000 genes in the human genome, as currently indicated, this would alter these figures to one true association every 1,500 genes studied. And the likelihood of an association with a P value of 0.05 arising by chance is more than 98%. Extrapolating this further, to increase the likelihood of a true positive to more than 95%, a P value of 0.00001 is needed. Clearly, the assumption that one polymorphism can fully assess the role of one gene is not valid and another estimate has set a P value of 0.00000005 for a genome-wide scan for association with a 95% probability of no false positives (Risch and Merikangas 1996). While the chances of identifying a positive association can be increased by examining strong candidates, it must

be admitted that the probability of identifying a novel true gene using an association approach alone is very slim.

The older methods for correcting for multiple testing, e.g. Bonferroni, are overly conservative, and other methods based on false discovery rates (Benjamini and Yekutieli 2001) and Monte Carlo approaches (Lin 2004) are being adopted in both genome-wide association studies and microarray expression experiments. As more information on psychoactive drug effects and gene function becomes available, Bayesian approaches to prior probabilities can be incorporated into statistical analysis (Greenland 2001). In the end, integration of information from clinical studies, pharmacology and basic molecular biology into genetic epidemiology will allow hypotheses with higher prior probability to be tested (Greenland *et al.* 2004).

Towards definitive studies. In the final analysis, definitive studies will depend on the ability to DNA re-sequence thousands of 'whole' human genomes, thereby eliminating the risk of selecting limited DNA-sequence variations for analysis in the hope that they carry sufficient information to answer the key biomedical questions (see Future Direction in Research).

In the meanwhile, there are a number of issues to be tackled with respect to studies of genetic influences in psychoactive drug use, effects and addiction, not least of which is the continuation and establishment of suitable human cohort studies or 'case' collections that combine detailed phenotyping, psychosocial and other exposures with consent for genetic analysis. One worry for the indirect association approach is the recent recognition that meiotic recombination 'hot spots', which are inaccessible to LD approaches, are associated with gene-conversion events (Jeffreys and May 2004) which could themselves be causal variants.

Population substructure. It is well understood that population stratification for both allele frequencies and disease prevalence can lead to false-positive results in case-control association studies, as well as to false negatives, the failure to detect real effects (Pritchard and Donnelly 2001). As study sizes grow (both in terms of sample sizes and the number of markers tested), even very small amounts of population substructure can theoretically cause serious difficulties for case-control studies (Marchini *et al.* 2004, Freedman *et al.* 2004), if not recognised and corrected for (Devlin and Roeder 1999, Devlin *et al.* 2004). Similar issues apply to cryptic relatedness, the phenomenon whereby cases may be more closely (though still very distantly) related than controls (e.g. Devlin and Roeder 1999). One way of circumventing population stratification problems is to study the differential transmission of alleles to affected offspring using parent-child trios. Such transmission disequilibrium tests (Speilman and Ewens 1996) add the complication of requiring parental DNA samples and have been regarded as being relatively underpowered statistically. However, there are recent indications that exhaustive allelic transmission disequilibrium tests can provide a valuable approach to genome-wide association studies exploiting LD to detect association with both common and rare causal variants (Lin *et al.* 2004). One is still left with actually defining which variants in the region are contributing to disease susceptibility or resistance.

Meta-analyses. Genetic association studies testing functional variants in candidate genes now represent a huge research industry. Because of the difficulties of assembling large, well-defined case collections or phenotypically-rich cohorts, these studies may be too statistically underpowered to detect small effects and often produce conflicting results. A secondary industry has sprung up, namely meta-analysis, where comparable studies are combined in a systematic way to produce valuable composite results (Munafo and Flint 2004). With cheaper genotyping, there is a current move towards gene-based analysis in which all common variation within a candidate gene is considered jointly. This is repeated in replication studies, although subsequent meta-analyses pose significant statistical challenges (Neale and Sham 2004)

3.1.4 Gene–environment interactions

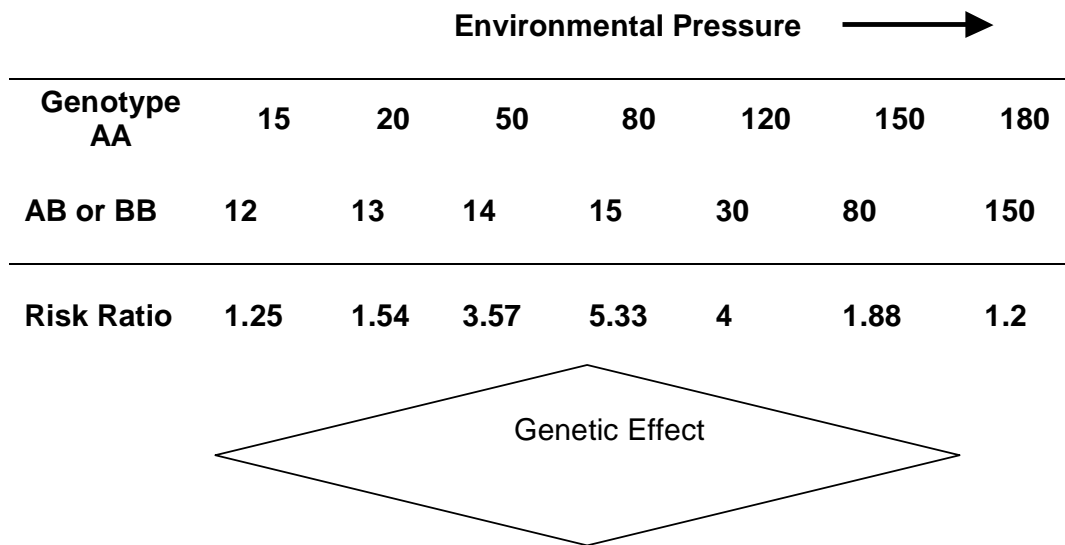
Given that the ultimate aim is to understand evolved developmental and regulatory responses as they relate to vulnerability to addiction and drug effects, the analysis of gene–environment interactions (GEI) has to be an integral part of future research. Designing and executing suitable studies is a huge challenge, given that 'genetic effects' may be strongly conditional on environmental pressures. Figure 2, originally based on the differential risk, by CYP1A1A genotype, of lung cancer with increasing lifetime cigarette-smoking dose (Nakachi *et al.* 1991), illustrates one possible scenario. The point is that the genetic effect in terms of risk could peak at a moderate level of exposure, while all genotypes are 'overwhelmed' at higher levels (Nakachi *et al.* 1991) and low exposure is barely sufficient to bring out the genetic differences (Hung *et al.* 2003). Whatever the exact population dynamics of the GEI, in many situations stratifying by exposure should help reveal genetic associations, provided the study has sufficient statistical power. This is not only a matter of sample size, but also of the quality of the measures used, since the ability to detect GEI increases as measurement error is reduced. For continuous traits, a sample size of 10,000 provides sufficient power using precise measures (Wong *et al.* 2003). It is generally, and rightly, assumed that collecting reliable measures of exposure and outcomes across the full range will be more feasible if done prospectively within a general population cohort. Such cohorts of the required size of ~10,000 are feasible, as demonstrated by the Avon Longitudinal Study of Parents and Children (ALSPAC) (Golding *et al.* 2001).

Where one could postulate from existing biological insights that an exposure might only have an adverse outcome in those with a particular genotype, the combined study of exposure and genotype will increase statistical power. However, there tends to be a general assumption that individual genetic variants will only contribute a low or modest risk and situations where a known environmental risk will only apply to those with a specific genotype will be 'unlikely to be frequently encountered in the study of complex disease' (Clayton and McKeigue 2001). In these circumstances it is argued that for adequate statistical power to detect modest risk ratios, the case-control design is more feasible than the cohort design (Clayton and McKeigue 2001). However, results over the last few years have shown this to be over pessimistic. There is now clear evidence that investigating interactions

between measured genes and measured environments can be a powerful route to ‘genetic’ discovery using cohorts (Moffitt *et al* 2005). Three studies from the Dunedin cohort in New Zealand (n~1000) have discovered measured GEI in mental disorders, and the first two findings have already been replicated by others (Moffitt *et al* 2005). The latest study demonstrates the moderation of the effect of adolescent-onset cannabis use on adult psychosis by a functional variant in the catechol-o-methyltransferase (COMT) gene (Caspi *et al* 2005).

Figure 2: One model of how the size of the genetic effect is contingent on the level of environmental pressure

In this population of 10,000, 9.29% die from lung cancer due to cigarette smoking. Overall, two-thirds of these cases have the AA genotype (risk ratio ~2). However, the risk ratio varies according to the lifetime dose of cigarettes, peaking at 5.33 with moderate exposure. This scenario is developed from data on the effect of a CYP1A1 variant on smoking-related lung cancer (Nakachi *et al.* 1991) where the 'genetic effect' became less with the heaviest exposure. The left-hand side of the curve is supported by the minimal (if any) effect of this CYP1A1 variant on lung cancer risk in non-smokers where exposure (?environmental tobacco smoke) is minimal (Hung *et al.* 2003).



3.1.5 Gene expression studies

Gene expression results are heavily dependent on the tissue being examined and, for much of the research into drug effects on the brain, tissue from a specific region of the brain is needed. For this reason, much current and future research will be on experimental animals, usually rodents, but also other mammalian species, and invertebrates such as fruit flies, or worms, since some of the effects of drugs on gene expression are at fundamental

levels of organisation common across the entire animal kingdom.¹ Gene chips can be used directly to study the influence of abused drugs on gene expression. Typically, gene chips contain several thousand genes and expression studies identify changes in a few dozen of them. Such mass screening is subject to errors and any changes observed require confirmation using more rigorous techniques. Sophisticated publicly available databases (e.g. webQTL; <http://www.webqtl.org>) allow researchers to search for behavioural traits associated with genes identified by microarray analyses.

Although it is an important first step, such knowledge does not provide an understanding of the mechanisms whereby changes in the expression of one or more genes may contribute to the behavioural change that lies at the heart of addiction, and it is likely to take many years of research before we understand the causal relationships between gene expression and cellular function, between cellular function and organ function, and organ function and whole organism, which will allow us to understand the relationship between gene expression and behaviour.

We have tended to write about genes as inherited material that contributes to, or even determines the occurrence of, behavioural traits, including addictive behaviour. However, genes do not only form the 'blueprint' for behaviour, but are themselves part of the mechanism whereby experience influences bodily, brain and behavioural function. Addictive drugs themselves influence brain plasticity and behaviour by altering the patterns of gene expression, turning on hitherto silent genes, while silencing others. Using microarray techniques,² it is possible to study treatment-induced changes in the expression of thousands of genes simultaneously, a method called gene expression profiling. In the short term, the expression of many different genes is altered by a single administration of a drug. In a recent study, we found a single dose of alcohol to alter the expression of over 140 genes in the midbrain of mice, nearly 2% of the genes tested. Some of these genes are transcription factors that in turn control the expression of other genes, some of them perhaps permanently.

Gene array (microarray; gene chip) techniques study changes in the abundance of mRNA in the tissue under investigation. While variations in mRNA will tell us something about which genes are being expressed in the tissue as a result of an experimental manipulation (e.g. feeding alcohol), gene expression at the mRNA level does not correlate well with either the level of the protein that it encodes, or with cellular function. As a result of post-transcription editing of the mRNA, a number of different proteins may be formed, each potentially with a different cellular function. Proteomics (the

¹ Although there are now several studies of the effects of drugs of abuse on gene expression in *Drosophila*, which may tell us something about the kinds of genes influenced by drugs, it must be noted that regulation of gene expression differs in some respects between mammals and invertebrates. On the other hand, it is technically easier, and perhaps ethically more acceptable, to create directed mutations in single genes in flies, so that hypotheses regarding the functions of individual genes in drug-related behaviour can be more efficiently tested. Most importantly, addiction is largely a behavioural disorder, and invertebrates offer little opportunity to model genomic influences on human behaviour.

² Microarrays contain a large number of an organism's genes individually gridded on to an area less than 2cm square. By spotting extract of a tissue on to the array, those genes that are currently active can be detected using a rapid automated screening procedure.

study of the protein make-up of a tissue) uses various techniques, such as mass spectrometry, to investigate protein levels, but none of them are able to capture the entire wealth of protein expression with the efficiency that gene array studies capture gene expression. Studies of protein expression are usually more limited in their extent, and more likely to be hypothesis-driven, than gene array studies. Techniques such as MALDI-TOF mass spectrometry are more akin to gene array studies, in that the experimenter need not have prior hypotheses as to the nature of the proteins altered by drug experience. But even these allow a sampling of only a subset of proteins (those that are not attached or imbedded in cellular membranes, precisely the proteins that are most likely to interact directly with drugs). Both proteomic and microarray methods are insensitive in identifying changes in levels of expression of those genes that are most active, or those that express at very low levels, and it is usually an arbitrary judgement whether, say, a two-fold increase or decrease in expression of one gene is considered more or less important biologically than a difficult-to-resolve 1.2-fold increase in the expression of another gene. These are difficult issues implicit in the field, and it may take considerable time to resolve them. Finally, current techniques study alterations in gene expression in whole organs, or regions of the brain, both in humans and in animal studies. Yet we know that such tissue samples are likely to be made up of many classes of cell (e.g. neurones and glia) so that some biologically important changes in expression in, say, neurones, may be undetectable against a background of gene expression in glia. When we consider the various functions of different neuronal types, each with its own distinct pattern of gene expression, the technical difficulty of the task facing us becomes clear. How drugs of abuse induce long-term changes in neuronal and other organ function that contribute to their pathological consequences, as well as to future propensity to take drugs, is an area for future research.

3.2 Illustrative examples of potential confounds in genetic analysis

It is important to appreciate that all genetic linkage and association studies end up with a similar 'end game'; namely deciding which of several genetic variants in and around a gene is actually causing the difference between people's vulnerability to addiction, organ damage, or response to treatment. If the variants are in strong LD, the question cannot be answered with association studies alone. Direct functional studies are needed using a combination of animal experiments and human cells or tissues. As described elsewhere, variation in DRD2 is of interest in both nicotine and alcohol addiction and their treatment. To this end studies have focused on the Taq1A polymorphism of the DRD2 gene, a C>T substitution located in a non-coding region of the DRD2 locus, which has been suggested to affect DRD2 availability in post-mortem striatal samples (Noble *et al.* 1991; Thompson *et al.* 1997). There is also evidence from *in vivo* studies for an association between the A1 (T) allele and lower-mean relative-glucose metabolic rate in dopaminergic regions in the human brain (Noble *et al.* 1997), and PET studies have indicated that this allele is also associated with low receptor density (Pohjalainen *et al.* 1998; Jönsson *et al.* 1999). However, recent work shows that the Taq1A site is, in fact, an amino-acid-changing single nucleotide polymorphism in a previously unrecognised protein kinase gene (ANKK1)

near the DRD2 locus (Neville *et al.* 2004). The two genes and therefore probably their variants are in LD, so the question now becomes: is the relevant causal change in the gene product from ANKK1 (e.g. the Taq1A site itself) or in the DRD2 gene where it just happens to be in strong LD with the Taq1A site? Resolving this type of problem will depend on functional studies of the specific genes and their variants in both animal and human studies.

Where exactly comparable animal models are not available for experimental studies, there are still approaches that can be used with human material. Methods are being developed to detect the effect of specific haplotypes on gene expression (Knight *et al.* 2003). Furthermore, genetic constructs incorporating 'risk' and 'non-risk' haplotypes into human cell lines can be made for functional analysis (Bochukova *et al.* 2003, Inoue *et al.* 2004).

Another issue that is particularly important in addiction is the possibility that the same gene variant is affecting both initiation behaviour and its subsequent biological effects. Alcohol drinking is considered to be a risk factor for oesophageal cancer, and exposure to high levels of acetaldehyde, the principle metabolite of alcohol, is hypothesised to be responsible for the increased cancer risk. The ability to metabolise acetaldehyde is encoded by the aldehyde dehydrogenase (ALDH2) gene, which is polymorphic in some populations. An individual's genotype at this locus may influence their oesophageal cancer risk through two mechanisms, first through influencing alcohol intake and second through influencing acetaldehyde levels. A meta-analysis of studies looking at the ALDH2 genotype and oesophageal cancer found that risk was reduced among *2*2 homozygotes OR = 0.35 (95%CI 0.17–0.71) and increased among heterozygotes OR = 3.06 (95%CI 2.55–3.67) relative to *1*1 homozygotes (Lewis and Davey Smith 2005). This provides strong evidence that alcohol intake increases the risk of oesophageal cancer and that individuals whose genotype results in lower alcohol intake (due to the intense side-effects) are thus protected. It also illustrates how the interpretation of genetic associations (in this case, in opposite directions) is relatively easy once the metabolic pathway is well understood. Just imagine trying to make sense of this, if the biology was unknown, from the knowledge that one *2 allele gives the highest risk and two *2 alleles give the lowest risk. It also shows the danger of relying entirely on allele-based, as opposed to genotype-based, statistical tests, particularly where the only model examined is an increasing risk from one to two alleles.

4 CURRENT STATE OF PLAY

4.1 Alcohol

4.1.1 Vulnerability to alcohol dependence

4.1.1.1 Family and twin studies

'Ebrii gignunt ebrios', or 'drunkards beget drunkards', is quoted by Robert Burton in *The Anatomy of Melancholy* (1625) and attributed to the Greek historian Plutarch (Burton 1972). Dating back to AD 110, it emphasises the long-standing recognition that alcohol dependence runs in families and is

hereditary. Interestingly, the original source for this quote invites an environmental interpretation, a timely reminder not to overemphasise the role of genetics (Plutarch 110).

Such observations have been given scientific credibility by formal family studies. In her review of 39 family studies, representing the families of 6,251 alcoholics and 4,083 non-alcoholics, Cotton (1979) reported that an alcoholic was six times more likely than a non-alcoholic to report parental alcoholism, although between 47% and 82% of alcoholics did not come from families in which one or both parents were alcoholics. Furthermore, the rates of alcoholism are significantly higher in relatives of individuals with a diagnosis of alcoholism (15.3%) than in non-alcoholic controls (8.7%)(Guze *et al.* 1986). And the odds of developing alcohol dependence increase with the proximity and number of affected relatives such that the risk is increased by 167% in individuals with both a first- and second-degree relative affected, 86% in those with an affected first-degree relative, and 45% among those with a second- or third-degree affected relative (Dawson, Harford and Grant 1992).

While these family studies are consistent with genetic influence, they do not represent proof, and further support has been derived from adoption studies. These natural experiments provide the opportunity to study individuals reared by unrelated parents. The earliest report was a small negative study by Roe and Burks. However, the subsequent three major adoption series provide strong evidence for the action of genetic factors in males, with weaker support in females (Bohman 1978; Bohman 1981; Bohman, Sigvardsson and Cloninger 1981; Cadoret *et al.* 1985; Cadoret and Gath 1978; Cadoret, Troughton and O'Gorman 1987; Cloninger, Bohman and Sigvardsson 1981; Goodwin *et al.* 1973; Goodwin *et al.* 1974; Goodwin *et al.* 1977a; Goodwin *et al.* 1977b; Roe and Burks 1945; Sigvardsson, Bohman and Cloninger 1996; Yates *et al.* 1996). Furthermore, there was no correlation between drinking behaviour in the adoptees and alcoholism in the adoptive parents and thus no evidence of a protective effect conferred by being raised away from the biological parent (Goodwin *et al.* 1974; Goodwin *et al.* 1977b). Thus, this increased rate of alcoholism in adoptees who have a biological parent who is alcoholic provides strong evidence for a significant genetic contribution to the development of alcoholism.

Further support and an estimate of heritability can be derived from twin studies. In essence, twin studies compare the rates of alcohol dependence in monozygotic and dizygotic twins. If a condition has a significant genetic component, provided the environmental factors making them similar or different are approximately the same, the greater concordance in monozygotic twins should be due to their greater genetic similarity. The results are by and large consistent with the view that genes contribute to familiarity and that alcoholism has a significant genetic component. Three basic approaches have been adopted: proband ascertainment with co-twin follow-up; the use of archival records with population-based twin registers; and clinical assessment using population-based twin registers (Prescott 2001). Estimates of heritability using these approaches range widely from 0.0 to 0.98 with typical figures of 0.5 for males and 0.25 for females (Allgulander, Nowak and Rice 1991; Caldwell and Gottesman 1991; Gurling, Murray and Clifford 1981; Gurling, Oppenheim and Murray 1984; Heath *et al.* 1997; Hrubec and Omenn 1981;

Kaij 1960; Kendler *et al.* 1992; Kendler *et al.* 1997; Koskenvuo *et al.* 1984; McGue, Pickens and Svikis 1992; Pickens *et al.* 1991; Prescott, Aggen, and Kendler 1999; Reed *et al.* 1996; Romanov *et al.* 1991; True *et al.* 1996).

An analogous approach in animals is the comparison of the addiction-related behaviours of inbred strains, whose individual members are essentially genetically identical. Some strains of mice, selected for qualities independent of addiction liability, such as the C57Bl/6 strain, willingly drink alcohol (Fuller 1964; Phillips *et al.* 1998), and may represent animal genetic models of some human types of excess alcohol consumption, while others such as the DBA/2 strain consume practically no alcohol. Several strains of rat have been purposely bred by selective breeding of the animals in a random population that, for example, consume high (or low) amounts of alcohol (Browman, Crabbe and Li 2000). Such selective breeding leads, over the generations, to strains with marked differences for the selected trait, providing good evidence that several aspects of alcohol-related behaviour are genetically determined. Such traits include sensitivity to alcohol, preference for and aversion to alcohol, and withdrawal severity. In the case of several mouse strains with high alcohol preference, and rats selected for high alcohol preference, the behaviour is associated with low functionality of the brain's serotonin systems, consistent with observations in humans. Within mouse strains, the tendency to drink alcohol, and the severity of acute alcohol withdrawal, were negatively correlated, suggesting that related genes may underlie both abuse and dependence (Metten *et al.* 1998), though it is important to acknowledge that these genes may influence alcohol-related behaviours as a consequence of broader behavioural influences. Thus, a cross-strain comparison revealed that strains that were capable, in response to a tone signal, of inhibiting an ongoing behaviour directed at obtaining food, also showed low ethanol consumption (Logue *et al.* 1998). Crabbe (2003) points out that a genetic predisposition to severe withdrawal, and a good ability to inhibit a prepotent behaviour, may lead some mouse strains not to consume alcohol when it is freely available.

4.1.1.2 Linkage and association studies

In spite of the likely genetic complexity of alcohol dependence, two recent linkage studies performed in the United States have reported positive findings and interestingly, several positive linkage regions contain potential candidate genes (see below) (Long *et al.* 1998; Reich *et al.* 1998). The smaller of the two enhanced the possibility of identifying linkage by using an ethnically homogeneous sample selected from the American Indian population. Linkage was reported in two chromosomal regions, namely chromosomes 4p and 11p. The best evidence for linkage was with a marker, D11S1984, on chromosome 11 and there was also good evidence for linkage with D4S3242 on chromosome 4. The linkage region on chromosome 4 is close to the β 1 GABA (gamma-aminobutyric acid) receptor gene (GABRB1), while that for chromosome 11 is close to the genes encoding the dopamine D4 receptor and tyrosine hydroxylase.

The larger, multi-centre study, COGA (Collaborative Study on the Genetics of Alcoholism) analysed genetic linkage in 105 multigenerational families

identified by centres in the United States (Reich *et al.* 1998). The COGA sample is largely composed of Caucasians (approximately 74%) with smaller numbers of African-Americans (approximately 17%) and Hispanics (6%). The study reports suggestive evidence of linkage on chromosomes 1 and 7, with a protective locus on chromosome 4 near the alcohol dehydrogenase (ADH) gene cluster. Following these initial reports, there have been rigorous re-analyses of the COGA data comparing definitions of phenotype and methods of analysis (Birznieks *et al.* 1999; Chen *et al.* 1999b; Comuzzie and Williams 1999; Curtis, Zhao and Sham 1999; Lin, Irwin, and Wright 1999; Turecki, Rouleau and Alda 1999). However, the original findings have not been fully confirmed in the replication sample (Hesselbrock *et al.* 2001).

The first reported genetic association study firmly established the DRD2 as a strong candidate gene. There followed both successful and failed attempts to replicate this finding and more robust association methods have not supported the original report (Edenberg *et al.* 1998). This highlights one of the fundamental problems with the association approach, i.e. that it is highly prone to false positives, and some argue that the chances of finding a false positive greatly outweigh that of a true positive result (Buckland 2001).

The most robust and convincing association findings have been reported with the alcohol-metabolising enzymes, primarily in oriental populations. Most ethanol elimination occurs via oxidation to acetaldehyde and acetate and this is mostly catalysed by ADH and aldehyde dehydrogenase 2 (ALDH2) (Yin and Agarwal 2001).

The ADH genes are clustered on the long arm of chromosome 4 and in oriental populations the frequencies of the high-velocity genetic variants of ADH₂ and ADH₃ are significantly decreased in alcoholics when compared with controls (Chen *et al.* 1996; Higuchi *et al.* 1995; Higuchi *et al.* 1996; Maezawa *et al.* 1995; Nakamura *et al.* 1996; Shen *et al.* 1997; Tanaka *et al.* 1997; Thomasson *et al.* 1991; Thomasson *et al.* 1994). These genetic variants are associated with a difference of up to 40-fold in the V_{max}, the limiting velocity of the reaction that metabolises ethanol to acetaldehyde, in the ADH₂ alleles. However, these associations have been less consistently reported in other populations (Borras *et al.* 2000; Ehlers *et al.* 2001; Gilder, Hodgkinson and Murray 1993; Whitfield *et al.* 1998). Despite this, the fact that these enzymes are clustered in one of the COGA linkage regions indicates that they may also be important determinants in non-oriental populations (Reich *et al.* 1998). As the greater difference in V_{max} is exhibited within the ADH₂ genetic variants, the apparent involvement of the ADH₃ locus may be due to linkage disequilibrium between these two genes as they are closely associated within the cluster (Osier *et al.* 1999). This suggestion is also supported by the lack of association between ADH₃ and alcohol dependence in Caucasian populations and a multiple logistic regression study of ALDH2, ADH₂ and ADH₃ in a Chinese population (Borras *et al.* 2000; Chen *et al.* 1999a; Couzigou *et al.* 1990; Gilder, Hodgkinson, & Murray 1993). At intoxicating levels of alcohol, ADH₄ a class II enzyme may account for up to 40% of ethanol oxidation. This gene has also been localised to the 4q22 ADH cluster and a functional polymorphism has been reported in the promoter region that doubles the promoter activity, a region involved in gene transcription or expression (Edenberg, Jerome and Li 1999).

ALDH2, the enzyme responsible for the majority of acetaldehyde oxidation, has been mapped to the long arm of chromosome 12. It exists in two forms that differ dramatically in activity and this is the result of a single nucleotide, or base, difference in exon 12 of the gene sequence (Yoshida *et al.* 1985). The enzymatic activity is virtually reduced to zero by this change. Following alcohol exposure, individuals carrying this low-activity variant experience high levels of acetaldehyde, with consequent unpleasant and aversive effects including pronounced facial flushing. This is a similar response to that produced by Antabuse, a drug used to aid the maintenance of abstinence. Not surprisingly, this low-activity variant protects against alcohol dependence (Chen *et al.* 1996; Higuchi *et al.* 1995; Maezawa *et al.* 1995; Shen *et al.* 1997; Thomasson *et al.* 1991; Yoshida 1992). However, the low-activity variant is rare in many races, including western Europeans, and does not play a significant protective role in such populations (Goedde *et al.* 1992).

The parsimonious interpretation of these associations between alcohol dependence and genetic variants in the alcohol-metabolising enzymes is that they exert their influence through an effect on levels of acetaldehyde. Thus, low-activity variants of ADH, namely ADH₂¹ and possibly ADH₃², and the high-activity 'wild type' allele of ALDH2, both result in lower levels of this aversive compound and this predisposes individuals to alcohol-dependence syndrome. Conversely, protection against alcohol dependence is afforded by high-activity variants of ADH and low-activity variants of ALDH2.

Less robust findings have also been reported using the association approach, primarily implicating genes within the dopaminergic, serotonergic and GABA systems as reported in the review by Dick and Foroud (2003).

4.1.2 Vulnerability to alcohol effects

In addition to vulnerability to addiction, genetic factors are also important in the development of addiction-related damage. It is striking that some individuals with severe alcohol-induced liver disease (ALD) present early in their drinking career, while this complication does not feature in others with more extensive drinking histories. Thus, while up to two-thirds of cases of cirrhosis in the United Kingdom are caused by alcohol abuse, only 8–30% of long-term abusers develop this condition (Grant, Dufour and Harford 1988; Saunders *et al.* 1981; Saunders and Williams 1983; Sherman and Williams 1994). This variation is in part related to genetic factors, demonstrated by twin studies reporting an increased concordance rate for ALD in monozygotic, when compared with dizygotic, twins (Hrubec and Omenn 1981). Thus, the development of this complication represents a complex interaction of genes and environment, which occurs during a process of disease development and progression. The search for the genes that underlie this genetic predisposition has primarily focused on the enzymes involved in alcohol metabolism, including ADH, ALDH2 and the cytochrome P450IIE1, part of the microsomal ethanol-oxidising system (MEOS) (Ball *et al.* 1995; Bataller, North and Brenner 2003; Hayashi, Watanabe and Kawajiri 1991). While some studies have supported a role for these genes in ALD, the findings have not been consistently replicated. Other genes that have also been implicated include those of the cytokine system, ApoE and superoxide dismutase, an antioxidant

(Degoul *et al.* 2001; Giraud *et al.* 1998; Grove *et al.* 1997; Grove *et al.* 2000; Iron *et al.* 1994; Takamatsu *et al.* 2000). Genetic factors have also been implicated in alcohol withdrawal symptoms, including fits and delirium, pancreatitis and Wernicke-Korsakoff syndrome (Hanck, Schneider and Whitcomb 2003; Heap *et al.* 2002; Matsushita *et al.* 2000; Muramatsu *et al.* 1997; Wernicke *et al.* 2002).

In summary, there is great and growing interest in the elucidation of genetic vulnerability to addiction-related damage. However, in common with those studies that address the predisposition to dependence itself, there is a lack of robust findings.

4.1.3 Genetics of treatment response for alcohol dependence

Interest in the role of DRD2 was initially focused on vulnerability to alcohol dependence and other addictions but has now been extended to include treatment response. Thus, the response to bromocriptine, a dopamine agonist, was compared in alcoholics, subdivided by DRD2 genotype. Those carrying the A1 allele, previously implicated in predisposition to alcoholism, demonstrated a greater response in terms of both craving and anxiety. Furthermore the lack of response reported by those homozygous for the A2 allele was such that this study raised the real possibility that this treatment could be targeted on the basis of a simple genetic test (Lawford *et al.* 1995). Despite such an important finding there do not appear to be any published attempts at replication. More recently, treatment response to naltrexone has been reported to be associated with genetic variation in the μ -opioid receptor (Oslin *et al.* 2003).

4.1.4 Gene expression studies and alcohol

Genes provide the blueprint that specifies and limits the organism's response to drugs, and also the means by which drugs induce long-term changes in the organism that affect its future behaviour, perhaps including its future predisposition to take drugs. Thus, although we are born with a certain complement of genes inherited from our parents, at any given time in development, many of these genes are switched off, so the proteins they encode are not synthesised and play no part in the organism's physiology. Inactive genes are switched on, and active genes switched off (or, more precisely, their activity is increased or decreased) at determined times in development. Gene expression is also regulated by experience, including exposure to drugs.

There is currently a large effort to understand which genes are activated and inactivated by particular drugs, and under which circumstances, and the mechanisms whereby such induction and suppression of gene activity occurs. Methods used for such studies include northern blots, differential display, real-time reverse transcriptase–polymerase chain reaction (RT-PCR), and *in situ* hybridisation. More recently, the development of DNA microarrays has facilitated the screening of the genome for actively-expressed genes. Using such methods, over 600 genes have been identified that are regulated in response to alcohol and may contribute to alcohol abuse and alcoholism.

Many of these genes contribute to signalling pathways that are already known to be influenced by alcohol. Thus, we know that alcohol affects neurotransmission by facilitating GABA_A receptors, by blocking glutamatergic N-methyl-D-aspartate (NMDA) receptors, and by increasing the release of opioid peptides. In keeping with this knowledge, a number of genes encoding subunits of GABA_A receptors, subunits of NMDA receptors, opiate receptors and opiate peptide transmitters, as well as genes encoding proteins involved in other neurotransmitter systems (including dopamine and serotonin) have been identified in animal studies as being regulated by ethanol. A second group of alcohol-regulated genes includes a number of transcription factors, including NkappaB, which themselves regulate the expression of other genes (see Worst and Vrana 2005 for review), and a third group includes various genes encoding proteins likely to be involved in cellular metabolic housekeeping functions that are influenced by alcohol. However, a wide array of other genes have also been identified from diverse biochemical pathways, only some of which have a clear relationship to alcohol's effects. It should be emphasised that there is considerable confusion in the literature at present, with different studies reporting different patterns of changes in gene expression. Different laboratories have used different means of administering alcohol, for different time periods, and these differences undoubtedly contribute to the range of findings. It will be some years before we have a clear appreciation of the most salient effects of alcohol on gene expression that are relevant for alcohol abuse, dependence, and toxicity.

Analysis of rat strains with high and low preference for alcohol has revealed that the high-preference strain has lower levels of serotonin innervation in brain regions including the hippocampus; higher levels of serotonin 1B receptors; and lower levels of opioid μ -receptors (Strother *et al.* 2001; Wong *et al.* 1993; Zhou *et al.* 1994). More recently, using microarray technology, Edenberg and colleagues (2005) have identified a series of genes that differ between alcohol-preferring and non-preferring rats. These include the preferring strain having increased expression of the GABA_A receptor α 1 subunit, but lower expression of the enzyme Gad1 which converts glutamate to the neurotransmitter GABA, perhaps indicating lower GABA function, and consistent with the human data reviewed above. However, a number of other genes also differ between the strains, including enzymes involved in aldehyde metabolism, as well as in cellular growth and maintenance, cell survival, and development as well as neurotransmission. In a recent presentation, Edenberg reported that in different brain areas, more than 240 genes differed between the strains. There is clearly much scope here for a genetic influence on ethanol preference in these strains.

In human post-mortem studies of alcoholics, a striking finding has been changes in genes in pathways associated with myelination of neurones (Lewohl *et al.* 2000; Mayfield *et al.* 2002). This is consistent with our knowledge of the loss of white matter in alcoholism. Nevertheless, a lack of agreement is also found among the several studies of gene expression in human alcoholic post-mortem tissue. Worst and Vrana (2005) compare three important reports (Lewohl *et al.* 2000; Mayfield *et al.* 2002; Sokolov *et al.* 2003) which together identify changes in expression of a few hundred genes in the brains of alcoholics. Strikingly, only six genes are identified in all three

reports. Likewise, a comparison of nine published reports of gene expression in various biological systems, including human alcoholic post-mortem tissue, rodent models of high alcohol consumption, and cultured human neuroblastoma cells exposed to alcohol, showed only 13 genes identified by two studies, and no genes in common between any three studies.

4.2 Nicotine

4.2.1 *Vulnerability to nicotine dependence*

4.2.1.1 Family and twin studies

Family studies demonstrate an increased rate of nicotine dependence in relatives of an individual similarly affected, which is consistent with the operation of genetic factors (Niu *et al.* 2000). Adoption studies provide further evidence for a genetic contribution to smoking (Osler *et al.* 2001). Estimates of this genetic contribution can be derived from twin studies. The heritability of smoking initiation ranges from 37% to 84% for women and from 28% to 84% in males. The heritability of smoking persistence varies between 53% and 71% for women and 52% to 69% for males (Hall, Madden and Lynskey 2002).

4.2.1.2 Linkage and association studies

Three linkage studies have attempted to identify genetic loci implicated in tobacco dependence. Two of these used the COGA families, recruited for the study of alcohol dependence as discussed above, and the third used 130 families recruited from Christchurch, New Zealand. These studies reported little overlap in findings, so regions on almost half of the chromosomes have been implicated; including chromosomes 2, 4, 5, 6, 9, 10, 14, 15, 16, 17 and 18 (Bergen *et al.* 1999; Duggirala, Almasy and Blangero 1999; Straub *et al.* 1999).

Furthermore, association studies have implicated genes involved in nicotine metabolism including cytochrome P450 2A6 (CYP2A6), 2B6 (CYP2B6) and 2E1 (CYP2E1), genes involved in the monoamine systems including the dopamine receptor 1 (DRD1), DRD2 described previously, dopamine transporter (DAT), dopamine beta-hydroxylase (DBH), monoamine oxidase A (MAOA), tyrosine hydroxylase (TH) and serotonin transporter (5HTT) along with the cholecystokinin (CCK) gene (Munafa *et al.* 2004; Tyndale 2003).

4.2.2 *Vulnerability to tobacco toxicity*

In nicotine dependence, attention has focused on elucidating the genes implicated in cancers and coronary heart disease (Bartsch *et al.* 2000; Humphries *et al.* 2001; Wang and Mahaney 2001). Prompted by the conversion of carcinogens originating from tobacco to DNA-reactive metabolites by cytochrome P450 enzymes, Bartsch and colleagues reviewed the effects of genetic variants in these enzymes, alone and in combination with other detoxifying enzymes. They reported an increased risk for cancers of the lung, oesophagus, head and neck associated with some cytochrome P450 variants. In addition, they describe a particularly potent combination of genotypes in CYP1A1 and GSTM1 that results in an increased risk for lung

cancer and was also associated with shortened post-operative survival (Goto *et al.* 1996; Kawajiri *et al.* 1996).

4.2.3 Pharmacogenetics of smoking cessation

Response to nicotine replacement therapy has been reported to be more effective in carriers of the A1 allele of the DRD2 gene (Johnstone *et al.* 2004). In another study, treatment response was also associated with genetic variation in the mu opioid receptor (Lerman *et al.* 2004). Treatment response to bupropion has also been reported to be associated with DRD2 – women carrying the A1 allele were more likely to report stopping taking this medication because of side-effects and at 12 months were more likely to report smoking, although this latter finding did not reach significance (Swan *et al.* 2004).

4.2.4 Gene expression studies and smoking

Less information is available on the consequences of smoking or nicotine treatment on gene expression than for alcohol. In a rat study (Li *et al.* 2004), as with similar studies using other abused drugs, some 200–300 genes were modulated in different brain regions, and following different periods of exposure. Within these genes, however, at any particular time, only a handful showed changes in expression in more than two brain regions, and several were up-regulated in one region but down-regulated in another. The dynamic complexity of these changes makes interpretation of the contribution of any particular changes impossible at this stage of knowledge. An added complexity is that nicotine administration early in life may have additional effects (see below).

4.2.5 Developmental 'programming' and smoking

One way of exploring developmentally sensitive periods in humans is to look at the effect of prenatal exposure through maternal smoking, as well as childhood onset of smoking. However, such studies need appropriate longitudinal designs that can deal with the main confounders. There is a growing body of evidence suggesting that maternal tobacco consumption during pregnancy may have specific, negative effects on a range of behavioural outcomes expressed in childhood and adolescence, including smoking (Brook, Brook and Whiteman 2000; Day *et al.* 2000; Fergusson, Horwood and Lynskey 1993; Griesler, Kandel and Davies 1988; Fergusson, Woodward and Horwood 1998; Cornelius *et al.* 2000; Kandel *et al.* 1994). Two recent studies (Oncken *et al.* 2004; Roberts *et al.* in press) have suggested that maternal smoking during pregnancy interacts with offspring sex i.e. only female offspring demonstrate an association between maternal smoking during pregnancy and smoking initiation.

Perinatal administration of nicotine produces a broad spectrum of effects on brain development, including inhibition of DNA synthesis, altered ornithine decarboxylase activity, altered neurotransmitter function, and significant alterations in cortical morphogenesis (Oliff and Gallardo 1999). Evidence also suggests that prenatal nicotine exposure may influence subsequent nicotine

preference in periadolescent offspring mice (Klein *et al.* 2003). In a separate study (Abreu-Villaça *et al.* 2004a, 2004b) prenatal nicotine exposure in rats reduced the up-regulation of nAChRs evoked by adolescent nicotine but worsened the cholinergic hypoactivity during withdrawal. Moreover, prenatal nicotine has also been reported to be associated with up-regulated nicotinic receptor mRNAs in neonate rats (Frank *et al.* 2001). Similar effects of perinatal nicotine exposure on nAChR gene expression have been reported in primates (Slotkin *et al.* 2004).

Recent evidence suggests that adolescence may represent a critical developmental period, characterised by enhanced neurobehavioural vulnerability to nicotine (Adriani *et al.* 2003). In this study, adult rats, pretreated with nicotine during periadolescence, showed significantly increased gene expression of the alpha-5, alpha-6 and beta-2 subunits of nAChR, thus enhancing the reinforcing efficacy of nicotine in a self-administration paradigm. In rats, nicotine exposure during adolescence dose-dependently down-regulated levels of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) GluR2/3 subunits in the striatum, which might result in reduced neurobehavioural plasticity. Comparable exposure during adulthood had opposite effects (Adriani and Laviola 2004). This is of substantial theoretical and clinical importance, given the increasing interest in nicotine replacement therapy for smoking cessation among adolescents, although it will be some time before we are able to integrate these kinds of observation into our understanding of addictions.

4.3 Other psychoactive substances

4.3.1 Family and twin studies

Early studies established elevated family history of drug abuse in the siblings of drug-abusing probands. Von Knorring *et al.* (1983) used the adoption method to show probable genetic transmission of substance abuse. The adoption studies of Cadoret (Cadoret *et al.* 1986) provided formal evidence for a genetic influence on drug abuse. More recent family studies have shown that family history of drug abuse or dependence is a potent risk factor for drug abuse, and that both general and drug-specific risk factors exist (Merikangas *et al.* 1998; Bierut *et al.* 1998).

Twin studies have confirmed the role of heritability on the risk of drug-abuse disorders. A series of small twin studies provided evidence in favour of heritability (Grove *et al.* 1990; Gynther *et al.* 1995; Jang, Livesley and Vernon 1995; Pickens and Svikis 1991). For example, Pedersen (1981) found heritability of 0.28 for the abuse of tranquillisers, and Pickens (Pickens and Svikis 1991; Pickens *et al.* 1991) found the heritability of drug abuse, as defined by DSM-III criteria, to be 0.31 for males and 0.22 for females. This work has been extended to the analysis of large-scale, population-based samples of twins, which has provided detailed information on the probable role of genes and environment in vulnerability to drug-abuse disorders in general and to the abuse of specific classes of drug (Kendler 2001). One problem with twin, family and adoption studies is that the level and type of drug abuse will vary over time. For example, a substantial increase in cocaine and crack use took place during the 1980s whereas heroin use fell (Lowinson,

Ruiz and Millman 1992). This must be borne in mind when interpreting twin studies. Heritability is not fixed but is dependent on environment, and exposure tends to maximise heritability.

Data from the Vietnam era veteran twin studies (VET) indicates that opiate abuse has additive genetic component of 43%, a unique environmental effect of 31% and a non-additive genetic effect of 26%, indicating high heritability for opiate abuse and dependence (Tsuang *et al.* 2001). Abuse of cannabis, stimulants, sedatives and Phencyclidine (PCP) and psychedelics all showed evidence for heritability, with a strong likelihood of substantial shared genetic vulnerability across classes of drug.

Genetic vulnerability to drug abuse appears to be composed of two components, one specific to the drug (for example, drug-specific pharmacogenetic factors) and one producing general vulnerability to drug abuse. More severe phenotypes (abuse and dependence) have higher heritability than use, which is influenced by shared as well as non-shared environment. Opiate abuse and dependence showed the lowest level of common genetic vulnerability with others drugs of abuse, at 50%, indicating that unique aspects of the pharmacology of opiates may be involved in vulnerability to abuse. Other twin studies, such as the Virginia Twin Registry and Drug Treatment Centre studies are consistent with these findings (Karkowski, Prescott and Kendler 2000; Kendler *et al.* 2000; Kendler, Karkowski and Prescott 1999; Kendler, *et al.* 1999; Kendler and Prescott 1998; van den Bree, Svikis and Pickens 1998).

In terms of animal studies, strain differences have been revealed in factors thought to reflect abuse of psychomotor stimulants such as cocaine, or opiates such as morphine. For example, the Lewis strain of rats exhibit greater conditioned place preference for cocaine (Kosten *et al.* 1994) and morphine (Guitart *et al.* 1992), but not for amphetamine (Stohr *et al.* 1998) than Fischer 344 rats and more readily acquire oral drug self-administration of opiates (Suzuki *et al.* 1992) and ethanol (Suzuki *et al.* 1988). Acquisition of intravenous morphine (Ambrosio *et al.* 1995) and cocaine (Kosten *et al.* 1997) self-administration also occurs more readily in the Lewis strain than in the Fischer 344 strain of rats.

4.3.2 Linkage and association studies

Molecular genetics has focused on candidate-gene approaches in humans, mainly examining polydrug-abusing populations, and on genetic linkage of opiate response traits in mice. Loci for morphine preference (Berrettini *et al.* 1994) and morphine analgesia (Crabbe *et al.* 1999) have been identified through quantitative trait linkage in the mouse. The locus on chromosome 10 for morphine preference contains the mu opioid receptor MOR. The most interesting finding in humans is the association between oral codeine abuse and the enzyme CYP2D6 (Tyndale, Droll and Sellers 1997). In general, there have not been replicated associations between drug abuse and specific genes, although many candidates from the dopamine system, drug metabolism pathways and drug-specific receptors have been examined. There are few studies examining the specific molecular genetics of cannabis, cocaine and amphetamine abuse and dependence, and research tends to

focus on polydrug abuse (reviewed in Ball and Collier 2002). In amphetamine use, there has been considerable interest in the genetic contribution to the development of methamphetamine-induced psychosis (Chen *et al.* 2004). Kreek *et al.* (2005) review the pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments.

Very recently it has been shown that the effect of adolescent-onset (but not adult-onset) cannabis use on adult psychosis is moderated by a very common variant in the COMT gene. Carriers of the COMT valine¹⁵⁸ allele were more likely to exhibit psychotic symptoms and develop schizophreniform disorder if they used cannabis (Caspi *et al.* 2005).

5 FUTURE DIRECTIONS IN RESEARCH

5.1 Experimental animal studies

We can anticipate that current approaches using gene expression will become more sophisticated as more effort is applied to defining the particular conditions under which gene expression studies are carried out and as we restrict our analyses to more and more discrete regions of the brain that can be more easily integrated with neuroscience and behavioural approaches. The development of hypotheses that are able to account for the relevance of changes in the expression of particular gene families, or of genes with related functions in biochemical pathways, will help to impose order on the recently acquired mass of data. The integration of gene expression data into QTL data from an earlier phase of genetic analysis will also help us to order the vast amount of information obtained from expression studies.

However, ordering the data on drug effects on gene expression is only the first step. Gene expression studies tell us little about exactly how drugs influence the expression of particular genes. Our rapidly advancing knowledge of how experiences in general come to regulate gene expression will impact on this area. There have been suggestions that drugs may alter chromatin organisation, as well as DNA methylation, in ways that could in principle account for control of expression (see epigenetics section below). Bottom-up approaches involving transgenic animals are also likely to become more sophisticated as it becomes increasingly feasible to influence the timing and the locality at which individual genes are suppressed or induced. The development of increasingly precise behavioural methods for studying mouse behaviour (since most mammalian transgenic studies are in mice), as well as the increasing availability of transgenic rats for which behavioural methods are already well established, will advance the field. The ability to manipulate gene expression in other ways such as the viral transfer of mammalian genes into discrete brain areas has already been developed and offers an additional tool to study gene effects. Thanos *et al.* (2001) reported use of an adenoviral vector to deliver the DRD2 gene into the nucleus accumbens of rats, previously trained to self-administer alcohol, and to assess whether DRD2 levels regulated alcohol preference and intake. Increases in DRD2 were associated with marked reductions in alcohol preference and alcohol intake of ethanol-preferring and outbred rat strains, which recovered as the DRD2

returned to baseline levels. This is the first evidence that overexpression of DRD2 reduces alcohol intake and suggests that high levels of DRD2 may be protective against alcohol abuse. The combination of viral transfer with knockout models to 'rescue' missing genes may provide a particularly convincing demonstration of the role of specific genes in drug abuse.

Future animal studies will benefit from discoveries about the nature of addictions derived from human research, and from neuroscience accounts of addiction. More precise descriptions of the psychological processes underlying addictive behaviour will allow animal psychologists to model these in animals, and study the consequences of gene manipulations on these defined processes. In genetics and genomic research, animal and human studies go hand in hand (see epigenetics section below).

5.2 Human studies

5.2.1 Clinical samples

Dependence is a complex disorder and is overlain with more levels of complexity than most. For example, the development of dependence requires exposure to a substance and this is in part determined by availability and acceptability, prior knowledge, anticipation and free will. Therefore sample selection, characterisation and numbers are vital to the success of genetic studies, which has considerable resource implications for such research. Thousands of carefully characterised samples are required, identified through both national and international collaboration. Internationally agreed assessment protocols, using both continuous and categorical measures, should be agreed and introduced to permit true meta-analyses. And while controls should be matched for the usual parameters such as age, sex, ethnicity and social status, perhaps the most important factor is exposure to substance in an environment similarly accepting of intoxication. For example, perhaps the best controls for studies of smoking are 'chippers,' who smoke a small number of cigarettes per day but do not progress to dependence. Ultimately, such matching is not possible as dependence is associated with its own pattern of consumption. Case-control studies would need to be supplemented with cohort studies.

5.2.2 Technology

The non-systematic nature of candidate-gene studies is being addressed by rapid developments in genotyping technology and the use of DNA pooling. DNA chip technology now permits the rapid examination of up to 100,000 single nucleotide polymorphisms in one reaction (<http://www.affymetrix.com/index.affx>). Another approach to reduce workload, and therefore cost, has been the pooling of DNA samples from multiple individuals as a means of screening genetic markers (Fisher *et al.* 1999; Hill *et al.* 1999). In the final analysis, definitive studies will depend on the ability to DNA re-sequence thousands of 'whole' human genomes, thereby eliminating the risk of selecting only some of the DNA-sequence variations for analysis in the hope that they carry sufficient information to answer the key biomedical questions, including those related to addiction. This definitive DNA-

sequencing will eventually be applied to cohort studies with sufficient phenotyping in breadth and depth (e.g. ALSPAC (www.alspac.bristol.ac.uk, Golding *et al.* 2001)) when genotyping costs fall compared to the cost of collecting phenotypic and exposure information on large numbers of study subjects. We don't know whether suitable genotyping technology will be delivered in 5, 10 or 15 years. The National Institutes of Health in the USA is aiming for the \$1,000 genome analysis by 2014.

5.2.3 Studying genes and environment in the context of development

The development of dependence is a complex interaction of multiple constitutional and environmental factors, including availability and acceptability, self-medication and personality, psychological and hereditary factors. The 'natural history of dependence' runs from substance-use initiation and maintenance, through the development of tolerance, dependence and all manner of consequences, to treatment attempts, relapse and ultimately recovery.

Perhaps one of the greatest challenges to research in this field is the interaction between genetic and environmental factors during the development of dependence. This is very much about the elucidation of the dash in 'nature–nurture'. Recently such interactions have been studied in a longitudinal sample from New Zealand, although this research has yet to study addiction directly. The Dunedin sample has followed a representative birth cohort of 1,037 subjects and the individuals have been assessed at 3, 5, 7, 9, 11, 13, 15, 18 and 21 years, the sample remaining virtually intact at 26 years. Using this sample the research team have examined the interaction of environmental factors with genes during the process of development. The first study described the moderating effect of a functional polymorphism in the gene encoding the neurotransmitter-metabolising enzyme monoamine oxidase A (MAOA) on the impact of maltreatment such that children with a genotype conferring high levels of MAOA expression were less likely to develop antisocial behaviour (Caspi *et al.* 2002). More recently, the same group has reported that the influence of life stress on depression is modulated by polymorphisms in the 5-HT transporter gene (Caspi *et al.* 2003). These effects need to be replicated in other cohorts. But the use of such well-characterised longitudinal samples seems to be delivering on the promise to identify gene and environmental interactions which occur in the development of a complex disorder or behaviour that could mask the genetic contribution if this is explored alone. All these projects will generate a vast amount of data and it is vital that they are supported by expert statistical and biometrical advice and the development of sophisticated methods of analysis.

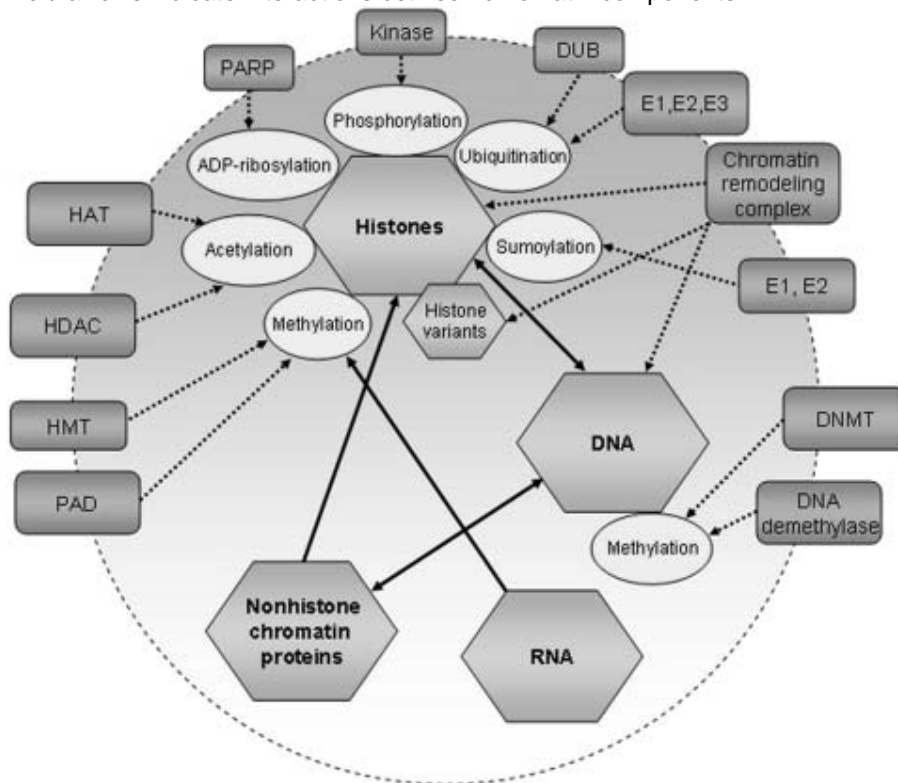
5.3 Developmental programming and epigenetics

An area of intense study at present, and highly relevant to psychoactive drug addiction and management in the future, is the longer-term modification of gene expression in the brain by psychological and drug exposures and the epigenetic processes that underpin developmental programming. At the molecular level, a gene–environment interaction is finally played out at the level of the chromatin. As Figure 3 shows, there are many molecules that

either associate directly with DNA or contribute to the complexes that induce changes at a gene promoter during gene expression.

Figure 3: The main epigenetic players illustrating the complexity of regulation of genetic response

Representation of chromatin components (hexagons), their modifications (ovals), and enzymatic complexes acting on them (quadrats). Dotted arrows denote enzymatic reactions. Bold arrows indicate interactions between chromatin components.



HAT: histone acetyl transferase, HDAC: histone deacetylase, DNMT: DNA methyl transferase, PAD: peptidylarginine deiminase, E1: ubiquitin activating enzyme, E2: ubiquitin conjugating enzyme, E3: ubiquitin ligase enzyme, E1 SUMO: SUMO activating enzyme.

Reproduced with permission from Roloff and Nuber 2005.

There are suggestions that drugs may alter chromatin organisation, as well as DNA methylation. As yet, a body of research from which a picture of psychoactive-drug induced epigenetic programming can be drawn has not emerged. Nevertheless, there are experimental hints from related fields of the way it is likely to go, such as maternal behaviour (to illustrate psychosocial effects) and schizophrenia research (to illustrate drug effects). In a rat model, the programming effect of early maternal behaviour on offspring defensive and stress responses has been shown to be mediated by epigenetic changes (histone acetylation and DNA methylation) in the promoter region of the glucocorticoid receptor gene in the brain of the offspring (Weaver *et al.* 2004). Changes in epigenetic state involve histone acetylation/deacetylation by histone deacetylase (HDAC) enzymes. The mood stabiliser and

anticonvulsant, valproic acid, has been shown to have HDAC-inhibiting properties (Gottlicher *et al.* 2001, Phiel *et al.* 2001). In a mouse model, valproic acid was able to influence (mRNA) expression in the RELN gene, which has reduced expression in post-mortem brains of patients with schizophrenia, in association with expected changes in acetylated histone H3 content (Tremolizzo *et al.* 2002). Sharma (2005) brings together the evidence on the epigenetic aspects of schizophrenia and then looks at the potential of 'chromatin therapeutics'. He regards valproic acid as a potential genome 'softener' to increase genome plasticity and thereby reinvigorate response in treatment-resistant cases.

One of the problems with exploring epigenetic effects in human clinical or epidemiological research is access to the appropriate tissue, before and after exposure, since many epigenetic effects are likely to be tissue-specific. Studies of prenatal exposure through maternal use of drugs would prove problematic in this respect, although there is one glimmer of hope. There is some experimental evidence in mice that maternal dietary manipulation with methyl donors in very early pregnancy can alter the methylation status of a type of DNA sequence called a transposable element, which in turn changes the expression of a nearby gene in the developing foetus – in this case, the *agouti* gene effecting coat colour (Waterland and Jirtle 2003). It is encouraging to note that the associated methylation patterns were established so early – and maintained with such high fidelity throughout development – that tissues derived from all three embryological layers had the same pattern, indicating that methylation patterns in lymphocytes might be useful in human studies.

The recognition of long-term and possibly permanent drug-induced epigenetic changes raises the question of whether this epigenetic change (or an acquired increased susceptibility to this change) can be transmitted to future generations.

5.3.1 Transgenerational effects

Various exposures, particularly to hormones and drugs, in one generation have been shown in experimental animal studies to produce effects in the next generations (Campbell and Perkins 1988). This can occur down both the female and male lines. A recent example is the effect of prenatal exposure to glucocorticoids in rats, with the offspring of male rats exposed *in utero* having a reduced birth weight and permanently increased activity of the key hepatic gluconeogenic enzyme, phosphoenolpyruvate carboxykinase (Drake, Walker and Seckl 2005). The transmitting mechanism is not known, but the possibility of transgenerational inheritance has to be entertained. Epigenetic or transgenerational inheritance has been demonstrated in mice, although this involved the transmission of an inherently variable epigenetic state rather than one triggered by a specific exposure (Rakyan *et al.* 2003).

At least two-generation cohort studies are needed to study transgenerational effects and this is not easy in humans. It is important to appreciate that the mother's genotype, insofar as it modulates her metabolism of drugs, for example, contributes to the foetal environment. Indeed the mother's developmentally 'programmed' metabolism captures information on some of

her own early exposures, and this could itself be transmitted to the developing foetus (Bateson *et al.* 2004). More remarkable is the emerging evidence from historical studies in Sweden that sperm seem to transmit ancestral nutritional information down the male line. For example, the nutrition of the paternal grandfather (specifically) in mid-childhood influences the mortality rate of his grandchildren (Bygren *et al.* 2001; Kaati, Bygren and Edvinsson 2002). There is unpublished evidence from the ALSPAC study that paternal onset of smoking in mid-childhood is associated with transgenerational effects on both gestational length and obesity in future sons (Pembrey and Golding). Whether these transgenerational effects represent epigenetic inheritance of an altered chromatin state (Pembrey 2002) or some other mechanism should be amenable to molecular analysis in the future.

6 THE IMPLICATIONS OF DEFINING GENETIC INFLUENCES IN DRUG USE FOR THE INDIVIDUAL AND SOCIETY

The identification of genes involved in addiction will elucidate the biological underpinning of the natural history of addiction from initiation, through maintenance, tolerance, dependence and the development of complications to recovery. It is anticipated that some genes will be common to addictive behaviours and others specific to a substance or behaviour. For example, genes implicated in reward mechanisms, such as the dopaminergic and opiate systems, may be relevant across substance and behaviour, whereas genes involved in drug metabolism are likely to be substance specific. Thus, the complex choreography that occurs between different genes and the environment, during a process of development, will be clarified.

Such an approach is not without its critics and some have argued that the potential benefits do not justify the investment, given that other treatment methods exist that may ultimately prove to be more effective than any gene-based strategies (Merikangas and Risch 2003).

6.1 The biological basis of addiction and treatment implications

Understanding the biological contributions to addiction will identify biological targets for the development of novel treatments, which are much needed in this field. For example, while medically assisted detoxification from alcohol is highly successful, the rates for maintenance of abstinence are poor, around 20% at 6–12 months (Sass *et al.* 1996). Such high relapse rates are very common across the addictive behaviour range. Furthermore, genotype–phenotype studies could identify subtypes of addiction, with implications for pharmacological, psychological, spiritual and social treatment strategies, and it may be possible to tailor the management of an individual on the basis of various prognostic indicators including a genetic test (Ball 2004; Ball and Collier 2002; Nuffield Council on Bioethics 1998).

6.2 Predisposition to addiction

If genes of major effect are found, it may be possible to refine an individual's risk of developing dependence using genetic data. Such predictive genetic testing is most applicable when a single gene confers a high risk, which, as we have seen, is an unlikely scenario in addiction. However, if several genes of relatively modest effect are identified, these could be combined in a multiplex reaction, or on a DNA chip, to provide an indication of risk. But the purpose of identifying individuals at increased risk is uncertain as most addictive behaviours confer no benefit and so the advice in general is to avoid use. Furthermore, the individual interpretation of such testing is unclear. A test result indicating low risk of dependence may encourage naïve individuals to experiment in the belief that they have no risk of progression to dependence. Similarly, a high result might support a fatalistic approach, either for the individual or for treatment services. Finally, it is unlikely that such risk alteration will be extended to population screening. The more genes are involved, the smaller will be the proportion of the population possessing most of the high-risk variants, making screening inefficient (Hall, Madden and Lynskey 2002; Hepple and Nuffield Council on Bioethics 2002; Nuffield Council on Bioethics 1998).

6.3 Vulnerability to the consequences of addictive behaviour

There may be a greater role for identifying the genes that predispose individuals to the complications of their addictive behaviour, although here again, a reported low risk of associated illness may encourage established users to persist in drug use in the belief that they are safe. This belief may, of course, be valid for some individuals and it is possible that we may eventually understand enough to test for this. As an example, future research may clarify the relationship between cannabis use and the early onset of schizophrenia. At present the jury is out on whether there is a causal link (Veen *et al.* 2004, Krebs, Goldberger and Dervaux 2005), but it is conceivable that there is a genetically definable subgroup for whom early smoking of cannabis does indeed constitute a significant risk factor for subsequent schizophrenia. Individuals may choose to take this test via the Internet, regardless of what the professional view is of such action. However, it has to be remembered that safety related to one aspect of substance use, for example, cancer in smoking, may confer a sense of security, not supported and sustained by the exposure to other risks, for example, cardiovascular complications (Hall, Madden and Lynskey 2002; Hepple and Nuffield Council on Bioethics 2002; Nuffield Council on Bioethics 1998).

In summary, the identification of genes involved in addiction, via their developmental interplay with the environment, will have a profound effect on the understanding of the field. This will inevitably have important implications for the management of patients from initiation, through tolerance, dependence, physical complications and treatment response. Genetic testing *per se* is likely to be of limited use (except perhaps in selecting medical treatments) and further extending such risk modification to population

screening would seem even less credible. The pessimistic future portrayed in the film *GATTACA*, in which individuals are given a percentage chance of developing addiction, pre-implantation screening of foetuses excludes this risk and role in society is determined by a DNA test, is highly unlikely to be fulfilled. However, in looking forward to this exciting future, the important moral and ethical lessons of the past should be heeded and the implications considered and widely debated (Müller-Hill 1998). While developments in this arena may influence public policy, perhaps the most profound effect is likely to be on the public attitude to addiction.

7 THE BSAD QUESTIONS

What will be the psychoactive substances of the future?

The contribution of genomics to this question will be indirect but could be important. Neuropharmacological understanding (to which genomics-based studies will contribute) might be able to predict the addictive potential of new substances and thereby aid the search for safer substitutes. Separating the pathways leading to addiction from other, therapeutic, pharmacological effects might reveal new targets for the development of drugs with defined effects. It has to be recognised that introducing new or existing drugs as novel approaches to serious disorders, as with the development of chromatin therapeutic agents like valproic acid (see above), also introduces a new agent for abuse. The addition of valproic acid as an 'enhancer' in psychoactive substance abuse, for example, could be highly dangerous.

What are the effects of using psychoactive substances?

We expect the application of genetics and genomics to make a big contribution to our understanding of the effects of using psychoactive substances, particularly addiction. The consequences of taking drugs for gene expression, leading to possibly permanently altered function, is a major part of understanding both the development of addictions and their long-term consequences for brain function, as well as adverse or beneficial effects on other organs. There is more than a remote chance that drug taking in childhood will have transgenerational consequences in terms of health, through both the female and the male line.

What mechanisms do we have to manage the use of psychoactive substances?

Despite the Government White Paper *Our Inheritance Our Future: Realising the potential of genetics in the NHS* (June 2003) raising the possible role of genetic profiling at birth in health management, we think 'genetic profiling' will have a limited role, if any, in the prevention of drug abuse. The individual genetic effects will be so contingent on other influences that the predictive power of genetic profiles to define vulnerability is likely to be limited. However, pharmacogenomics will lead to more refined, targeted treatment.

8 CONCLUSIONS

- Advances in affordable genotyping and gene expression and epigenetic analysis will not be the limiting factor. Many 'genes' with hitherto unknown function will be characterised.
- Animal research will also not be limiting, as we understand commonalities in the genes underlying addictive behaviours in humans and in animal models.
- The biggest challenge is applying these technologies and biological insights to research on the human population. To supplement clinical and family studies, the optimum design is a large contemporary (two-generation) pre-birth cohort study in which the full range of psychosocial and other environmental exposures have been documented prospectively and the full range of developmental outcomes are being measured.

9 References

- Abreu-Villaça Y, Seidler FJ, Slotkin TA. Does prenatal nicotine exposure sensitize the brain to nicotine-induced neurotoxicity in adolescence? *Neuropsychopharmacology* 2004a; 29: 1440–1450.
- Abreu-Villaça Y, Seidler FJ, Tate CA, Cousins MM, Slotkin TA. Prenatal nicotine exposure alters the response to nicotine administration in adolescence: effects on cholinergic systems during exposure and withdrawal. *Neuropsychopharmacology* 2004b; 29: 879–890.
- Adriani W, Laviola G. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. *Behav Pharmacol* 2004; 15: 341–352.
- Adriani W, Spijker S, Deroche-Gamonet V *et al.* Evidence for enhanced neurobehavioral vulnerability to nicotine during periadolescence in rats. *J Neurosci* 2003; 23: 4712–4716.
- Allgulander C, Nowak J, Rice JP. Psychopathology and treatment of 30,344 twins in Sweden. II. Heritability estimates of psychiatric diagnosis and treatment in 12,884 twin pairs. *Acta Psychiatr Scand* 1991; 83: 12–15.
- Altmuller J, Palmer LJ, Fischer G, Scherb H, Wjst M. Genomewide scans of complex human diseases: true linkage is hard to find. *Am J Hum Genet* 2001; 69: 936–950.
- Ambrosio E, Goldberg SR, Elmer GI. Behavior genetic investigation of the relationship between spontaneous locomotor activity and the acquisition of morphine self-administration behavior. *Behav Pharmacol* 1995; 6: 229–237.
- Ball D. Genetic approaches to alcohol dependence. *Br J Psychiatry* 2004; 185: 449–451.
- Ball D, Collier D. Substance misuse. In: McGuffin P *et al.* (eds). *Psychiatric genetics and genomics*. Oxford: Oxford University Press, 2002.
- Ball DM, Sherman D, Gibb R *et al.* No association between the c2 allele at the cytochrome P450IIIE1 gene and alcohol induced liver disease, alcohol Korsakoff's syndrome or alcohol dependence syndrome. *Drug Alcohol Depend* 1995; 39: 181–184.
- Bartsch H, Nair U, Risch A, Rojas M, Wikman H, Alexandrov K. Genetic polymorphism of CYP genes, alone or in combination, as a risk modifier of tobacco-related cancers. *Cancer Epidemiol Biomarkers Prev* 2000; 9: 3–28.
- Bataller R, North KE, Brenner DA. Genetic polymorphisms and the progression of liver fibrosis: a critical appraisal. *Hepatology* 2003; 37: 493–503.
- Bateson P, Barker D, Clutton-Brock T *et al.* Developmental plasticity and human health. *Nature* 2004;430:419–421.
- Benjamini Y, Yekutieli D. The control of the false discovery rate in multiple testing under dependency. *The Annals of Statistics* 2001; 29:1165–1188.

Bergen AW, Korczak JF, Weissbecker KA, Goldstein AM. A genome-wide search for loci contributing to smoking and alcoholism. *Genet Epidemiol* 1999; 17 Suppl 1: S55–S60.

Berrettini WH, Ferraro TN, Alexander RC, Buchberg AM, Vogel WH. Quantitative trait loci mapping of three loci controlling morphine preference using inbred mouse strains. *Nat Genet* 1994; 7: 54–58.

Bierut LJ, Dinwiddie SH, Begleiter H *et al.* Familial transmission of substance dependence: alcohol, marijuana, cocaine, and habitual smoking: a report from the Collaborative Study on the Genetics of Alcoholism. *Arch Gen Psychiatry* 1998; 55: 982–988.

BioNews 286: 29/11/04. <http://www.BioNews.org.uk>

Birznieks G, Ghosh S, Watanabe RM, Mitchell BD. The effect of phenotype variation on detection of linkage in the COGA data. *Genet Epidemiol* 1999; 17 Suppl 1: S61–S66.

Blackburn EH. Structure and function of telomeres. *Nature* 1991; 350: 569–573.

Bochukova EG, Jefferson A, Francis MJ, Monaco AP. Genomic studies of gene expression: regulation of the Wilson's disease gene. *Genomics* 2003; 81:531–542.

Bohman M. Some genetic aspects of alcoholism and criminality. A population of adoptees. *Arch Gen Psychiatry* 1978; 35: 269–276.

Bohman M. The interaction of heredity and childhood environment: some adoption studies. *J Child Psychol Psychiatry* 1981; 22: 195–200.

Bohman M, Sigvardsson S, Cloninger CR. Maternal inheritance of alcohol abuse. Cross-fostering analysis of adopted women. *Arch Gen Psychiatry* 1981; 38: 965-969.

Borras E, Coutelle C, Rosell A *et al.* Genetic polymorphism of alcohol dehydrogenase in Europeans: the ADH2*2 allele decreases the risk for alcoholism and is associated with ADH3*1. *Hepatology* 2000; 31: 984–989.

Botstein, D., and Risch, N. (2003). Discovering genotypes underlying human phenotypes: past successes for mendelian disease, future approaches for complex disease. *Nat Genet* 33 Suppl. 228–237.

Brook JS, Brook DW, Whiteman M. The influence of maternal smoking during pregnancy on the toddler's negativity. *Arch Pediatr Adolesc Med* 2000; 154: 381–385.

Browman KE, Crabbe JC, Li TK. Alcohol and genetics: new animal models. In: Bloom FE and Kupfer DJ (eds). *Psychopharmacology: a fourth generation of progress*. New York: Lippincott, Williams and Wilkins, 2000.

Buckland PR. Genetic association studies of alcoholism: problems with the candidate-gene approach. *Alcohol Alcohol* 2001; 36: 99–103.

Burton P. Commentary: Gene-environment interactions: fundamental yet elusive. *Int J Epidemiol* 2001; 30: 1040–1041.

Burton R. *The anatomy of melancholy*. London : Dent, 1972.

- Bygren LO, Kaati G, Edvinsson S. Longevity determined by ancestors' overnutrition during their slow growth period. *Acta Biotheoretica* 2001; 49:53–59.
- Cadoret RJ, Gath A. Inheritance of alcoholism in adoptees. *Br J Psychiatry* 1978; 132: 252–258.
- Cadoret RJ, Troughton E, O'Gorman TW. Genetic and environmental factors in alcohol abuse and antisocial personality. *J Stud Alcohol* 1987; 48: 1–8.
- Cadoret RJ, O'Gorman TW, Troughton E, Heywood E. Alcoholism and antisocial personality. Interrelationships, genetic and environmental factors. *Arch Gen Psychiatry* 1985; 42: 161–167.
- Cadoret RJ, Troughton E, O'Gorman TW, Heywood E. An adoption study of genetic and environmental factors in drug abuse. *Arch Gen Psychiatry* 1986; 43: 1131–1136.
- Caldwell, C. B. and Gottesman, I. I. Sex differences in the risk for alcoholism: a twin study. *Behav Genet* 1991; 21, 563.
- Campbell JH, Perkins P. Transgenerational effects of drug and hormonal treatments in mammals: a review of observations and ideas. *Prog Brain Res* 1988; 73: 535–553.
- Carlezon WA, Jr., Nestler EJ. Elevated levels of GluR1 in the midbrain: a trigger for sensitization to drugs of abuse? *Trends Neurosci* 2002; 25: 610–615.
- Carter N. As normal as normal can be? *Nature Genetics* 2004; 36: 931–932.
- Caspi A, McClay J, Moffitt TE *et al.* Role of genotype in the cycle of violence in maltreated children. *Science* 2002; 297: 851–854.
- Caspi A, Moffitt TE, Cannon M, McClay J, Murray R, Harrington H, Taylor A, Arseneault L, Williams B, Braithwaite A, Poulton R, Craig IW. Moderation of the effects of adolescent-onset cannabis use on adult psychosis by a functional polymorphism in the catecho-o-methyltransferase gene: longitudinal evidence of a gene x environment interaction. *Biol Psychiatry* 2005; 57: 1117–1127.
- Caspi A, Sugden K, Moffitt TE *et al.* Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science* 2003; 301: 386–389.
- Chen CC, Lu RB, Chen YC *et al.* Interaction between the functional polymorphisms of the alcohol- metabolism genes in protection against alcoholism. *Am J Hum Genet* 1999a; 65: 795–807.
- Chen CH, Finch SJ, Mendell NR, Gordon D. Comparison of empirical strategies to maximize GENEHUNTER lod scores. *Genet Epidemiol* 1999b; 17 Suppl 1: S103–S108.
- Chen CK, Hu X, Lin SK *et al.* Association analysis of dopamine D2-like receptor genes and methamphetamine abuse. *Psychiatr Genet* 2004; 14: 223–226.

Chen WJ, Loh EW, Hsu YP, Chen CC, Yu JM, Cheng AT. Alcohol-metabolising genes and alcoholism among Taiwanese Han men: independent effect of ADH2, ADH3 and ALDH2. *Br J Psychiatry* 1996; 168: 762–767.

Clayton D, McKeigue PM. Epidemiological methods in studying genes and environmental factors in complex diseases. *Lancet* 2001; 358: 1356–1360.

Cloninger CR, Bohman M, Sigvardsson S. Inheritance of alcohol abuse. Cross-fostering analysis of adopted men. *Arch Gen Psychiatry* 1981; 38: 861–868.

Comuzzie AG, Williams JT. Correcting for ascertainment bias in the COGA data set. *Genet Epidemiol* 1999; 17 Suppl 1:S109-S114.

Cornelius MD, Leech SL, Goldschmidt L, Day NL. Prenatal tobacco exposure: is it a risk factor for early tobacco experimentation? *Nicotine and Tobacco Research* 2000; 2: 45–52.

Cotton NS. The familial incidence of alcoholism: a review. *J Stud Alcohol* 1979; 40: 89–116.

Couzigou P, Fleury B, Groppi A, Cassaigne A, Begueret J, Iron A. Genotyping study of alcohol dehydrogenase class I polymorphism in French patients with alcoholic cirrhosis. The French Group for Research on Alcohol and Liver. *Alcohol Alcohol* 1990; 25: 623–626.

Crabbe JC. Finding genes for complex behaviors: progress in mouse models of the addictions. In: Plomin R, Defries JC, Craig IW and McGuffin P (eds). *Behavioral genetics in the postgenomic era*. Washington, DC: American Psychological Association, 2003.

Crabbe JC, Phillips TJ, Buck KJ, Cunningham CL, Belknap JK. Identifying genes for alcohol and drug sensitivity: recent progress and future directions. *Trends Neurosci* 1999; 22: 173–179.

Curtis D, Zhao JH, Sham PC. Comparison of GENEHUNTER and MFLINK for analysis of COGA linkage data. *Genet Epidemiol* 1999; 17 Suppl 1: S115–S120.

Dawson DA, Harford TC, Grant BF. Family history as a predictor of alcohol dependence. *Alcohol Clin Exp Res* 1992; 16: 572–575.

Day NL, Richardson GA, Goldschmidt L, Cornelius MD. Effects of prenatal tobacco exposure on preschoolers' behavior. *J Dev Behav Pediatr* 2000; 21: 180–188.

De Felipe C, Herrero JF, O'Brien JA *et al*. Altered nociception, analgesia and aggression in mice lacking the receptor for substance P. *Nature* 1998; 392: 394–397.

Degoul F, Sutton A, Mansouri A *et al*. Homozygosity for alanine in the mitochondrial targeting sequence of superoxide dismutase and risk for severe alcoholic liver disease. *Gastroenterology* 2001; 120: 1468–1474.

Devlin B, Roeder K. Genomic control for association studies. *Biometrics* 1999; 55: 997–1004.

Devlin B, Bacanu SA, Roeder K. Genomic Control to the extreme. *Nat Genet* 2004; 36: 1129–1130.

- Dick DM, Foroud T. Candidate genes for alcohol dependence: a review of genetic evidence from human studies. *Alcohol Clin Exp Res* 2003; 27: 868–879.
- DiFranza JR, Aligne CA, Weitzman M. Prenatal and postnatal environmental tobacco smoke exposure and children's health. *Pediatrics* 2004; 113: 1007–1015.
- Drake AJ, Walker BR, Seckl JR. Intergenerational consequences of fetal programming by in utero exposure to glucocorticoids in rats *Am J Physiol Regul Comp Physiol* 2005; 288.
- Duggirala R, Almasy L, Blangero J. Smoking behavior is under the influence of a major quantitative trait locus on human chromosome 5q. *Genet Epidemiol* 1999; 17 Suppl 1: S139–S144.
- Dywer T, Ponsonby A-L, Stankovich J, Blizzard L, Easteal S. Measuring environmental factors can enhance the search for disease causing genes? *J Epidemiol Community Health* 2004; 58: 613–615.
- Edenberg HJ, Jerome RE, Li M. Polymorphism of the human alcohol dehydrogenase 4 (ADH4) promoter affects gene expression. *Pharmacogenetics* 1999; 9: 25–30.
- Edenberg HJ, Foroud T, Koller DL *et al.* A family-based analysis of the association of the dopamine D2 receptor (DRD2) with alcoholism. *Alcohol Clin Exp Res* 1998; 22: 505–512.
- Edenberg HJ *et al.* Gene expression in the hippocampus of inbred alcohol-preferring and -nonpreferring rats. *Genes Brain Behav* 2005 Feb; 4(1): 20-30.
- Ehlers CL, Gilder DA, Harris L, Carr L. Association of the ADH2*3 allele with a negative family history of alcoholism in African American young adults. *Alcohol Clin Exp Res* 2001; 25: 1773–1777.
- Fergusson DM, Horwood LJ, Lynskey MT. Maternal smoking before and after pregnancy: effects on behavioral outcomes in middle childhood. *Pediatrics* 1993; 92: 815–822.
- Fergusson DM, Woodward LJ, Horwood LJ. Maternal smoking during pregnancy and psychiatric adjustment in late adolescence. *Arch Gen Psychiatry* 1998; 55: 721–727.
- Fisher PJ, Turic D, Williams NM *et al.* DNA pooling identifies QTLs on chromosome 4 for general cognitive ability in children. *Hum Mol Genet* 1999; 8: 915–922.
- Fisher RA. The limits of intensive production in animals. *Br Agr Bull* 1951; 4: 217–218.
- Frank MG, Srere H, Ledezma C, O'Hara B, Heller HC. Prenatal nicotine alters vigilance states and AchR gene expression in the neonatal rat: implications for SIDS. *Am J Physiol Regul Integr Comp Physiol* 2001; 280: R1134–R1140.
- Freedman ML, Reich D, Penney KL *et al.* Assessing the impact of population stratification on genetic association studies. *Nature Genetics* 2004; 36: 388–393.

- Freimer N, Sabatti C. The human phenome project. *Nature Genetics* 2003; 34:15–21.
- Fuller JL. Measurement of Alcohol Preference in Genetic Experiments. *J Comp Physiol Psychol* 1964; 57: 85–88.
- Gilder FJ, Hodgkinson S, Murray RM. ADH and ALDH genotype profiles in Caucasians with alcohol-related problems and controls. *Addiction* 1993; 88: 383–388.
- Giraud V, Naveau S, Betoulle D *et al.* [Influence of apolipoprotein E polymorphism in alcoholic cirrhosis]. *Gastroenterol Clin Biol* 1998; 22: 571–575.
- Goedde HW, Agarwal DP, Fritze G *et al.* Distribution of ADH2 and ALDH2 genotypes in different populations. *Hum Genet* 1992; 88: 344–346.
- Golding J, Pembrey M, Jones R, The ALSPAC Study Team. ALSPAC: The Avon Longitudinal Study of Parents and Children. I. Study methodology. *Paediatric and Perinatal Epidemiol* 2001;15:74–87.
- Goodwin DW, Schulsinger F, Hermansen L, Guze SB, Winokur G. Alcohol problems in adoptees raised apart from alcoholic parents. *Arch Gen Psychiatry* 1973; 28: 238–243.
- Goodwin DW, Schulsinger F, Knop J, Mednick S, Guze SB. Alcoholism and depression in adopted-out daughters of alcoholics. *Arch Gen Psychiatry* 1977a; 34: 751–755.
- Goodwin DW, Schulsinger F, Knop J, Mednick S, Guze SB. Psychopathology in adopted and nonadopted daughters of alcoholics. *Arch Gen Psychiatry* 1977b; 34: 1005–1009.
- Goodwin DW, Schulsinger F, Moller N, Hermansen L, Winokur G, Guze SB. Drinking problems in adopted and nonadopted sons of alcoholics. *Arch Gen Psychiatry* 1974; 31: 164–169.
- Goto I, Yoneda S, Yamamoto M, Kawajiri K. Prognostic significance of germ line polymorphisms of the CYP1A1 and glutathione S-transferase genes in patients with non-small cell lung cancer. *Cancer Res* 1996; 56: 3725–3730.
- Gottesman II, Gould TD. The endophenotype concept in psychiatry: etymology and strategic Intentions. *Am J Psychiatry* 2003; 160: 636–645.
- Gottesman II, Hanson DR. Human development: biological and genetic processes. *Annu Rev Psychol* 2005; 56:263–286.
- Gottlicher M, Minucci S, Zhu P *et al.* Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* 2001; 20: 6969–6978.
- Grant BF, Dufour MC, Harford TC. Epidemiology of alcoholic liver disease. *Semin Liver Dis* 1988; 8: 12–25.
- Greenland S. Sensitivity analysis, Monte Carlo risk analysis, and Bayesian uncertainty assessment. *Risk Analysis* 2001; 21: 579–583.
- Greenland S, Gago-Dominduez M, Castelao JE. Value of risk-factor ('black box') epidemiology. *Epidemiology* 2004; 15: 529–535.

- Griesler PC, Kandel DB, Davies M. Maternal smoking in pregnancy, child behaviour problems, and adolescent smoking. *Journal of Research on Adolescence* 1988; 8: 159–185.
- Grove WM, Eckert ED, Heston L, Bouchard TJ, Jr., Segal N, Lykken DT. Heritability of substance abuse and antisocial behavior: a study of monozygotic twins reared apart. *Biol Psychiatry* 1990; 27: 1293–1304.
- Grove J, Daly AK, Bassendine MF, Day CP. Association of a tumor necrosis factor promoter polymorphism with susceptibility to alcoholic steatohepatitis. *Hepatology* 1997; 26: 143–146.
- Grove J, Daly AK, Bassendine MF, Gilvarry E, Day CP. Interleukin 10 promoter region polymorphisms and susceptibility to advanced alcoholic liver disease. *Gut* 2000; 46: 540–545.
- Guitart X, Beitner-Johnson D, Marby DW, Kosten TA, Nestler EJ. Fischer and Lewis rat strains differ in basal levels of neurofilament proteins and their regulation by chronic morphine in the mesolimbic dopamine system. *Synapse* 1992; 12: 242–253.
- Guo SW. Gene-environment interaction and the mapping of complex traits: some statistical models and their implications. *Hum Hered* 2000; 50: 286–303.
- Gurling HM, Murray RM, Clifford CA. Investigations into the genetics of alcohol dependence and into its effects on brain function. *Prog Clin Biol Res* 1981; 69 Pt C: 77–87.
- Gurling HM, Oppenheim BE, Murray RM. Depression, criminality and psychopathology associated with alcoholism: evidence from a twin study. *Acta Genet Med Gemellol (Roma)* 1984; 33: 333–339.
- Guze SB, Cloninger CR, Martin R, Clayton PJ. Alcoholism as a medical disorder. *Compr Psychiatry* 1986; 27: 501–510.
- Gynther LM, Carey G, Gottesman II, Vogler GP. A twin study of non-alcohol substance abuse. *Psychiatry Res* 1995; 56: 213–220.
- Hall W, Madden P, Lynskey M. The genetics of tobacco use: methods, findings and policy implications. *Tob Control* 2002; 11: 119–124.
- Hanck C, Schneider A, Whitcomb DC. Genetic polymorphisms in alcoholic pancreatitis. *Best Pract Res Clin Gastroenterol* 2003; 17: 613–623.
- Hayashi S, Watanabe J, Kawajiri K. Genetic polymorphisms in the 5'-flanking region change transcriptional regulation of the human cytochrome P450IIE1 gene. *J Biochem (Tokyo)* 1991; 110: 559–565.
- Heap LC, Pratt OE, Ward RJ *et al*. Individual susceptibility to Wernicke-Korsakoff syndrome and alcoholism-induced cognitive deficit: impaired thiamine utilization found in alcoholics and alcohol abusers. *Psychiatr Genet* 2002; 12: 217–224.
- Heath AC, Bucholz KK, Madden PA *et al*. Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. *Psychol Med* 1997; 27: 1381–1396.

- Helgadottir A, Manolescu A, Thorleifsson G *et al.* The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. *Nat Genet* 2004; 36: 233–239.
- Hepple BA, Nuffield Council on Bioethics. *Genetics and human behaviour : The ethical context*. London : Nuffield Council on Bioethics, 2002.
- Hesselbrock V, Foroud T, Edenberg HJ, Nurnberger J, Jr., Reich T, Rice J. Genetics and Alcoholism: The COGA Project. In: Agarwal DP, Seitz HK (eds). *Alcohol in health and disease*. New York: Marcel Dekker, Inc., 2001: 103–124.
- Higuchi S, Matsushita S, Murayama M, Takagi S, Hayashida M. Alcohol and aldehyde dehydrogenase polymorphisms and the risk for alcoholism. *Am J Psychiatry* 1995; 152: 1219–1221.
- Higuchi S, Muramatsu T, Matsushita S, Murayama M, Hayashida M. Polymorphisms of ethanol-oxidizing enzymes in alcoholics with inactive ALDH2. *Hum Genet* 1996; 97: 431–434.
- Hill L, Craig IW, Asherson P *et al.* DNA pooling and dense marker maps: a systematic search for genes for cognitive ability. *Neuroreport* 1999; 10: 843–848.
- Horikawa Y, Oda N, Cox NJ *et al.* Genetic variation in the gene encoding calpain-10 is associated with type 2 diabetes mellitus. *Nat Genet* 2000; 26: 163–175.
- Hrubec Z, Omenn GS. Evidence of genetic predisposition to alcoholic cirrhosis and psychosis: twin concordances for alcoholism and its biological end points by zygosity among male veterans. *Alcohol Clin Exp Res* 1981; 5: 207–215.
- Humphries SE, Talmud PJ, Hawe E, Bolla M, Day IN, Miller GJ. Apolipoprotein E4 and coronary heart disease in middle-aged men who smoke: a prospective study. *Lancet* 2001; 358: 115–119.
- Hung RJ, Boffetta P, Brockmoller J. *et al.* CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a pooled analysis. *Carcinogenesis* 2003; 24: 875–882.
- Inoue R, Moghaddam KA, Ranasinghe M, Saeki Y, Chiocca EA, Wade-Martins R. Infectious delivery of the 132 kb CDKN2A/CDKN2B genomic DNA region results in correctly spliced gene expression and growth suppression in glioma cells. *Gene Ther* 2004; 11(15):1195–204.
- International HapMap Consortium. The International HapMap Project. *Nature* 2003; 426: 786–796.
- Iron A, Richard P, Pascual DZ, Dumas F, Cassaigne A, Couzigou P. Genetic polymorphism of apolipoprotein E in Caucasian alcoholic cirrhotics. *Alcohol Alcohol* 1994; 29: 715–718.
- Jang KL, Livesley WJ, Vernon PA. Alcohol and drug problems: a multivariate behavioural genetic analysis of co-morbidity. *Addiction* 1995; 90: 1213–1221.
- Jeffreys AJ, May CA. Intense and highly localized gene conversion activity in human meiotic crossover hot spots. *Nature Genetics* 2004; 36: 151–156.

- Johnstone EC, Yudkin PL, Hey K *et al.* Genetic variation in dopaminergic pathways and short-term effectiveness of the nicotine patch. *Pharmacogenetics* 2004; 14: 83-90.
- Jonsson EG, Nothen MM, Grunhage F *et al.* Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. *Mol Psychiatry* 1999; 4: 290–296.
- Kaati G, Bygren LD, Edvinsson S. Cardiovascular and diabetes mortality determined by nutrition during parents' and grandparents' slow growth period. *European Journal of Human Genetics* 2002; 10: 682–688.
- Kaj L. *Alcoholism in Twins*. Stockholm: Almqvist and Wiksell, 1960.
- Kandel DB, Wu P, Davies M. Maternal smoking during pregnancy and smoking by adolescent daughters. *Am J Public Health* 1994; 84: 1407–1413.
- Karkowski LM, Prescott CA, Kendler KS. Multivariate assessment of factors influencing illicit substance use in twins from female-female pairs. *Am J Med Genet* 2000; 96: 665–670.
- Kawajiri K, Eguchi H, Nakachi K, Sekiya T, Yamamoto M. Association of CYP1A1 germ line polymorphisms with mutations of the p53 gene in lung cancer. *Cancer Res* 1996; 56: 72–76.
- Kendler KS, Prescott CA. Cannabis use, abuse, and dependence in a population-based sample of female twins. *Am J Psychiatry* 1998; 155: 1016–1022.
- Kendler KS, Karkowski L, Prescott CA. Hallucinogen, opiate, sedative and stimulant use and abuse in a population-based sample of female twins. *Acta Psychiatr Scand* 1999; 99: 368–376.
- Kendler KS, Heath AC, Neale MC, Kessler RC, Eaves LJ. A population-based twin study of alcoholism in women. *JAMA* 1992; 268: 1877–1882.
- Kendler KS, Prescott CA, Neale MC, Pedersen NL. Temperance board registration for alcohol abuse in a national sample of Swedish male twins, born 1902 to 1949. *Arch Gen Psychiatry* 1997; 54: 178–184.
- Kendler KS, Karkowski LM, Corey LA, Prescott CA, Neale MC. Genetic and environmental risk factors in the aetiology of illicit drug initiation and subsequent misuse in women. *Br J Psychiatry* 1999; 175: 351–356.
- Kendler KS, Karkowski LM, Neale MC, Prescott CA. Illicit psychoactive substance use, heavy use, abuse, and dependence in a US population-based sample of male twins. *Arch Gen Psychiatry* 2000; 57: 261–269.
- Kendler KS, Prescott CA, Myers J, Neale MC. The structure of genetic and environmental risk factors for common psychiatric and substance use disorders in men and women. *Arch Gen Psychiatry* 2003; 60: 929–937.
- Kendler KS. Twin studies of psychiatric illness: an update. *Arch Gen Psychiatry* 2001; 58: 1005–1014.
- Klein LC, Stine MM, Pfaff DW, Vandenberg DJ. Maternal nicotine exposure increases nicotine preference in periadolescent male but not female C57B1/6J mice. *Nicotine Tob Res* 2003; 5: 117–124.

- Knight JC, Keating BJ, Rockett KA, Kwiatkowski DP. In vivo characterization of regulatory polymorphisms by allele-specific quantification of RNA polymerase loading. *Nature Genet* 2003; 33:469–475.
- Koskenvuo M, Langinvainio H, Kaprio J, Lonqvist J, Tienari P. Psychiatric hospitalization in twins. *Acta Genet Med Gemellol (Roma.)* 1984; 33: 321–332.
- Kosten TA, Miserendino MJ, Chi S, Nestler EJ. Fischer and Lewis rat strains show differential cocaine effects in conditioned place preference and behavioral sensitization but not in locomotor activity or conditioned taste aversion. *J Pharmacol Exp Ther* 1994; 269: 137–144.
- Kosten TA, Miserendino MJ, Haile CN, DeCaprio JL, Jatlow PI, Nestler EJ. Acquisition and maintenance of intravenous cocaine self-administration in Lewis and Fischer inbred rat strains. *Brain Res* 1997; 778: 418–429.
- Krebs M-O, Goldberger C, Dervaux A. Cannabis use and schizophrenia. *Am J Psychiatry* 2005; 162: 401–402.
- Kreek MJ, Bart G, Lilly C, LaForge KS, Nielsen DA. Pharmacogenetics and human molecular genetics of opiate and cocaine addictions and their treatments. *Pharmacol Rev* 2005; 57(1): 1-26.
- Kruglyak, L., and Nickerson, D. A. (2001). Variation is the spice of life. *Nat Genet* 27(3), 234–236.
- Lander ES. The new genomics: global views of biology. *Science* 1996; 274: 536–539.
- Lawford BR, Young RM, Rowell JA *et al.* Bromocriptine in the treatment of alcoholics with the D2 dopamine receptor A1 allele. *Nat Med* 1995; 1: 337–341.
- Lerman C, Wileyto EP, Patterson F *et al.* The functional mu opioid receptor (OPRM1) Asn40Asp variant predicts short-term response to nicotine replacement therapy in a clinical trial. *Pharmacogenomics J* 2004; 4: 184–192.
- Lewis and Davey Smith. Personal communication 2005.
- Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, Harris RA. Gene expression in human alcoholism: microarray analysis of frontal cortex. *Alcohol Clin Exp Res* 2000; 24: 1873–1882.
- Li MD, Kane JK, Wang J, Ma JZ. Time-dependent changes in transcriptional profiles within five rat brain regions in response to nicotine treatment. *Brain Res Mol Brain Res* 2004; 132: 168–180.
- Lin DY. An efficient Monte Carlo approach to assessing statistical significance in genomic studies. *Bioinformatics*. 2004; 28 September. Epub ahead of print.
- Lin S, Chakravarti A, Cutler D. Exhaustive allelic transmission disequilibrium tests as a new approach to genome-wide association studies. *Nature Genetics* 2004; 36: 1181–1188.
- Lin S, Irwin ME, Wright FA. A multiple locus analysis of the collaborative study on the genetics of alcoholism data set. *Genet Epidemiol* 1999; 17 Suppl 1: S229–S234.

Logue SF, Swartz RJ, Wehner JM. Genetic correlation between performance on an appetitive-signaled nosepoke task and voluntary ethanol consumption. *Alcohol Clin Exp Res* 1998; 22: 1912–1920.

Long JC, Knowler WC, Hanson RL *et al.* Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am J Med Genet* 1998; 81: 216–221.

Lowinson JH, Ruiz P, Millman RB. *Substance abuse: a comprehensive handbook*. Baltimore, Maryland, USA: Williams and Wilkins, 1992.

Maezawa Y, Yamauchi M, Toda G, Suzuki H, Sakurai S. Alcohol-metabolizing enzyme polymorphisms and alcoholism in Japan. *Alcohol Clin Exp Res* 1995; 19: 951–954.

Marchini J, Cardon LR, Phillips MS, Donnelly P. The effects of human population structure on large genetic association studies. *Nature Genetics* 2004; 36:512–517.

Matsushita S, Kato M, Muramatsu T, Higuchi S. Alcohol and aldehyde dehydrogenase genotypes in Korsakoff syndrome. *Alcohol Clin Exp Res* 2000; 24: 337–340.

Mayfield RD, Lewohl JM, Dodd PR, Herlihy A, Liu J, Harris RA. Patterns of gene expression are altered in the frontal and motor cortices of human alcoholics. *J Neurochem* 2002; 81: 802–813.

McGue M, Pickens RW, Sviki DS. Sex and age effects on the inheritance of alcohol problems: a twin study. *J Abnorm Psychol* 1992; 101: 3–17.

Merikangas KR, Risch N. Genomic priorities and public health. *Science* 2003; 302: 599–601.

Merikangas KR, Stolar M, Stevens DE *et al.* Familial transmission of substance use disorders. *Arch Gen Psychiatry* 1998; 55: 973–979.

Metten P, Phillips TJ, Crabbe JC *et al.* High genetic susceptibility to ethanol withdrawal predicts low ethanol consumption. *Mamm Genome* 1998; 9: 983–990.

Moffitt TE, Caspi A, Rutter M. Strategy for investigating interactions between measured genes and measured environments. *Arch Gen Psychiatry* 2005; 62: 473–481.

Morice E, Denis C, Giros B, Nosten-Bertrand M. Phenotypic expression of the targeted null-mutation in the dopamine transporter gene varies as a function of the genetic background. *Eur J Neurosci* 2004; 20: 120–126.

Mukherjee RAS, Hollins S, Abou-Saleh MT, Turk J. Low level alcohol consumption and the foetus. *BMJ* 2005;330: 375–376.

Mullikin J. Personal communication.

Müller-Hill B. *Murderous science: elimination by scientific selection of Jews, Gypsies, and others in Germany, 1933–1945*. Plainview, NY: Cold Spring Harbor Laboratory Press, 1998.

- Munafo M, Clark T, Johnstone E, Murphy M, Walton R. The genetic basis for smoking behavior: a systematic review and meta-analysis. *Nicotine Tob Res* 2004; 6: 583–597.
- Munafo MR, Flint J. Meta-analysis of genetic association studies. *Trends Genet* 2004; 20: 439–444.
- Muramatsu T, Kato M, Matsui T *et al.* Apolipoprotein E epsilon 4 allele distribution in Wernicke-Korsakoff syndrome with or without global intellectual deficits. *J Neural Transm* 1997; 104: 913–920.
- Murtra P, Sheasby AM, Hunt SP, De Felipe C. Rewarding effects of opiates are absent in mice lacking the receptor for substance P. *Nature* 2000; 405: 180–183.
- Nakachi K, Imai K, Hayashi S, Watanabe J, Kawajiri. Genetic susceptibility to squamous cell carcinoma of the lung in relation to cigarette smoking dose. *Cancer Research* 1991; 51: 5177–5180.
- Nakamura K, Iwahashi K, Matsuo Y, Miyatake R, Ichikawa Y, Suwaki H. Characteristics of Japanese alcoholics with the atypical aldehyde dehydrogenase 2*2. I. A comparison of the genotypes of ALDH2, ADH2, ADH3, and cytochrome P-450E1 between alcoholics and nonalcoholics. *Alcohol Clin Exp Res* 1996; 20: 52–55.
- Neale BM, Sham PC. The future of association studies: gene-based analysis and replication. *Am J Hum Genet* 2004; 75: 353–362.
- Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat* 2004; 23: 540–545.
- Niccol A. *Gattaca*. USA: Columbia Tristar/Jersey, 1997.
- Niu T, Chen C, Ni J *et al.* Nicotine dependence and its familial aggregation in Chinese. *Int J Epidemiol* 2000; 29: 248–252.
- Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Arch Gen Psychiatry* 1991; 48: 648–654.
- Noble EP, Gottschalk LA, Fallon JH, Ritchie TL, Wu JC. D2 dopamine receptor polymorphism and brain regional glucose metabolism. *Am J Med Genet* 1997; 74: 162–166.
- Nuffield Council on Bioethics. *Mental disorders and genetics: the ethical context*. London: Nuffield Council on Bioethics, 1998.
- Oliff HS, Gallardo KA. The effect of nicotine on developing brain catecholamine systems. *Front Biosci* 1999; 4: D883–D897.
- Oncken C, McKee S, Krishnan-Sarin S, O'Malley S, Mazure C. Gender effects of reported in utero tobacco exposure on smoking initiation, progression and nicotine dependence in adult offspring. *Nicotine Tob Res* 2004; 6: 829–833.
- Osier M, Pakstis AJ, Kidd JR *et al.* Linkage disequilibrium at the ADH2 and ADH3 loci and risk of alcoholism. *Am J Hum Genet* 1999; 64: 1147–1157.

- Osler M, Holst C, Prescott E, Sorensen TI. Influence of genes and family environment on adult smoking behavior assessed in an adoption study. *Genet Epidemiol* 2001; 21: 193–200.
- Oslin DW, Berrettini W, Kranzler HR *et al*. A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. *Neuropsychopharmacology* 2003; 28: 1546–1552.
- Pedersen N. Twin similarity for usage of common drugs. *Prog Clin Biol Res* 1981; 69 Pt C: 53–59.
- Pembrey ME. Time to take epigenetic inheritance seriously. *European Journal of Human Genetics* 2002; 10: 669–71.
- Pembrey ME. Genetic epidemiology: some special contributions of birth cohorts. *Paediatric and Perinatal Epidemiology* 2004; 18: 3–7.
- Pembrey and Golding. Personal communication.
- Phiel CJ, Zhang F, Huang EY, Guenther MG, Lazar MA, Klein PS. Histone deacetylase is a direct target of valproic acid, a potent anticonvulsant, mood stabilizer and teratogen. *J Biol Chem* 2001; 276: 36734–36741.
- Phillips TJ, Belknap JK, Buck KJ, Cunningham CL. Genes on mouse chromosomes 2 and 9 determine variation in ethanol consumption. *Mamm Genome* 1998; 9: 936–941.
- Pickens RW, Svikis DS. Genetic influences in human substance abuse. *J Addict Dis* 1991; 10: 205–213.
- Pickens RW, Svikis DS, McGue M, Lykken DT, Heston LL, Clayton PJ. Heterogeneity in the inheritance of alcoholism. A study of male and female twins. *Arch Gen Psychiatry* 1991; 48: 19–28.
- Plutarch. *The Training of Children*. 110.
- Pohjalainen T, Rinne JO, Nagren K *et al*. The A1 allele of the human D2 dopamine receptor gene predicts low D2 receptor availability in healthy volunteers. *Mol Psychiatry* 1998; 3: 256–260.
- Prescott CA. The genetic epidemiology of alcoholism. In: Agarwal DP, Seitz HK (eds). *Alcohol in health and disease*. New York: Marcel Dekker, 2001: 125–49.
- Prescott CA, Aggen SH, Kendler KS. Sex differences in the sources of genetic liability to alcohol abuse and dependence in a population-based sample of US twins. *Alcohol Clin Exp Res* 1999; 23: 1136–1144.
- Pritchard JK. Are rare variants responsible for susceptibility to complex disease? *Am J Hum Genet* 2001; 124–137.
- Pritchard JK, Donnelly P. Case-control studies of association in structured or admixed populations. *Theor Popul Biol* 2001; 60(3): 227–237.
- Rakyan VK, Chong S, Champ ME *et al*. Transgenerational inheritance of epigenetic states at the murine *Axin^{Fu}* allele occurs following maternal and paternal transmission. *Proc Nat Acad Sci* 2003; 100: 2538–2543.
- Reed T, Page WF, Viken RJ, Christian JC. Genetic predisposition to organ-specific endpoints of alcoholism. *Alcohol Clin Exp Res* 1996; 20: 1528–1533.

- Reich T, Edenberg HJ, Goate A *et al.* Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet* 1998; 81: 207–215.
- Ripley TL, Gadd CA, De Felipe C, Hunt SP, Stephens DN. Lack of self-administration and behavioural sensitisation to morphine, but not cocaine, in mice lacking NK1 receptors. *Neuropharmacology* 2002; 43: 1258–1268.
- Risch NJ. Searching for genetic determinants in the new millennium. *Nature* 2000; 405: 847–856.
- Risch N, Merikangas K. The future of genetic studies of complex human diseases. *Science* 1996; 273: 1516–1517.
- Roberts KH, Munafò MR, Rodriguez D *et al.* Longitudinal analysis of prenatal nicotine exposure on offspring's subsequent smoking behaviour. *Nicotine and Tobacco Research* in press.
- Rocha BA, Fumagalli F, Gainetdinov RR *et al.* Cocaine self-administration in dopamine-transporter knockout mice. *Nat Neurosci* 1998; 1: 132–137.
- Rockman MV, Wray GA. Abundant raw material for *cis*-regulatory evolution in humans. *Molecular Biology and Evolution* 2002; 19: 1991–2004.
- Roe A, Burks B. Adult adjustment of foster-children of alcoholic and psychotic parentage and the influence of the foster home. *Quarterly Journal of the Study of Alcohol* 1945.
- Roloff TC, Nuber UA. Chromatin, epigenetics and stem cells. *Eur J Cell Biol* 2005; 84(2-3): 123–135.
- Romanov K, Kaprio J, Rose RJ, Koskenvuo M. Genetics of alcoholism: effects of migration on concordance rates among male twins. *Alcohol Alcohol Suppl* 1991; 1: 137-140.
- Sass H, Soyka M, Mann K, Zieglgansberger W. Relapse prevention by acamprosate. Results from a placebo-controlled study on alcohol dependence. *Arch Gen Psychiatry* 1996; 53: 673–680.
- Saunders JB, Williams, R. The genetics of alcoholism: is there an inherited susceptibility to alcohol related problems? *Alcohol Alcohol* 1983; 18: 189–217.
- Saunders JB, Walters JR, Davies AP, Paton A. A 20-year prospective study of cirrhosis. *Br Med J (Clin Res Ed)* 1981; 282: 263–266.
- Scriver CR. After the genome – the phenome? *J Inherit Metab Dis* 2004; 27: 305–317.
- Sham P, McGuffin P. Linkage and association. In: McGuffin P, Owen M, Gottesman II (eds). *Psychiatric genetics and genomics*. Oxford: Oxford University Press, 2002: 55–73.
- Sharma RP. Schizophrenia, epigenetics and ligand-activated nuclear receptors: a framework for chromatin therapeutics. *Schizophrenia Research* 2005; 72: 79–90.

- Shen YC, Fan JH, Edenberg HJ *et al.* Polymorphism of ADH and ALDH genes among four ethnic groups in China and effects upon the risk for alcoholism. *Alcohol Clin Exp Res* 1997; 21: 1272–1277.
- Sherman DI, Williams R. Liver damage: mechanisms and management. *Br Med Bull* 1994; 50: 124–138.
- Sigvardsson S, Bohman M, Cloninger CR. Replication of the Stockholm Adoption Study of alcoholism. Confirmatory cross-fostering analysis. *Arch Gen Psychiatry* 1996; 53: 681–687.
- Sokolov BP, Jiang L, Trivedi NS, Aston C. Transcription profiling reveals mitochondrial, ubiquitin and signaling systems abnormalities in postmortem brains from subjects with a history of alcohol abuse or dependence. *J Neurosci Res* 2003; 72: 756–767.
- Sora I, Wichems C, Takahashi N *et al.* Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proc Natl Acad Sci USA* 1998; 95: 7699–7704.
- Speilman RS, Ewens WJ The TDT and other family-based tests for linkage disequilibrium and association. *American Journal of Human Genetics* 1996; 59: 983–989.
- Stephens DN, Mead AN, Ripley TL. Studying the neurobiology of stimulant and alcohol abuse and dependence in genetically manipulated mice. *Behav Pharmacol* 2002; 13: 327–45.
- Stohr T, Schulte Wermeling D, Weiner I, Feldon J. Rat strain differences in open-field behavior and the locomotor stimulating and rewarding effects of amphetamine. *Pharmacol Biochem Behav* 1998; 59: 813–818.
- Straub RE, Sullivan PF, Ma Y *et al.* Susceptibility genes for nicotine dependence: a genome scan and follow-up in an independent sample suggest that regions on chromosomes 2, 4, 10, 16, 17 and 18 merit further study. *Mol Psychiatry* 1999; 4: 129–144.
- Strother WN, Chernet EJ, Lumeng L, Li TK, McBride WJ. Regional central nervous system densities of delta-opioid receptors in alcohol-preferring P, alcohol-nonpreferring NP, and unselected Wistar rats. *Alcohol* 2001; 25: 31–38.
- Suzuki T, George FR, Meisch RA. Differential establishment and maintenance of oral ethanol reinforced behavior in Lewis and Fischer 344 inbred rat strains. *J Pharmacol Exp Ther* 1988; 245: 164–70.
- Suzuki T, George FR, Meisch RA. Etonitazene delivered orally serves as a reinforcer for Lewis but not Fischer 344 rats. *Pharmacol Biochem Behav* 1992; 42: 579–586.
- Swan GE, Valdes AM, Ring HZ, Khroyan TV, Jack LM, Ton CC *et al.* Dopamine receptor DRD2 genotype and smoking cessation outcome following treatment with bupropion SR. *Pharmacogenomics J* 2005; 5(1): 21–29.
- Takamatsu M, Yamauchi M, Maezawa Y, Saito S, Maeyama S, Uchikoshi T. Genetic polymorphisms of interleukin-1beta in association with the

- development of alcoholic liver disease in Japanese patients. *Am J Gastroenterol* 2000; 95: 1305–1311.
- Tanaka F, Shiratori Y, Yokosuka O, Imazeki F, Tsukada Y, Omata M. Polymorphism of alcohol-metabolizing genes affects drinking behavior and alcoholic liver disease in Japanese men. *Alcohol Clin Exp Res* 1997; 21: 596–601.
- Thanos PK, Volkow ND, Freimuth P *et al.* Overexpression of dopamine D2 receptors reduces alcohol self-administration. *J Neurochem* 2001; 78: 1094–1103.
- Thomasson HR, Edenberg HJ, Crabb DW *et al.* Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet* 1991; 48: 677–681.
- Thomasson HR, Crabb DW, Edenberg HJ *et al.* Low frequency of the ADH2*2 allele among Atayal natives of Taiwan with alcohol use disorders. *Alcohol Clin Exp Res* 1994; 18: 640–643.
- Thompson J, Thomas N, Singleton A *et al.* D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. *Pharmacogenetics* 1997; 7: 479–484.
- Tremolizzo L, Carboni G, Ruzicka WB *et al.* an epigenetic mouse model for molecular and behavioural neuropathologies related to schizophrenia vulnerability. *Proc Natl Acad Sci USA* 2002; 99: 17095–17100.
- True WR, Heath AC, Bucholz K *et al.* Models of treatment seeking for alcoholism: the role of genes and environment. *Alcohol Clin Exp Res* 1996; 20: 1577–1581.
- Tsuang MT, Bar JL, Harley RM, Lyons MJ. The Harvard Twin Study of Substance Abuse: what we have learned. *Harv Rev Psychiatry* 2001; 9: 267–279.
- Turecki G, Rouleau GA, Alda M. Family density of alcoholism and linkage information in the analysis of the COGA data. *Genet Epidemiol* 1999; 17 Suppl 1: S361–S366.
- Tyndale RF. Genetics of alcohol and tobacco use in humans. *Ann Med* 2003; 35: 94–121.
- Tyndale RF, Droll KP, Sellers EM. Genetically deficient CYP2D6 metabolism provides protection against oral opiate dependence. *Pharmacogenetics* 1997; 7: 375–379.
- van den Bree MB, Svikis DS, Pickens RW. Genetic influences in antisocial personality and drug use disorders. *Drug Alcohol Depend* 1998; 49: 177–187.
- Van Eerdewegh P, Little RD, Dupuis J *et al.* Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418: 426–430.
- Veen ND, Selton J-P, van der Tweed I, Feller WG, Hoek HW, Kahn RS. Cannabis use and age of onset of schizophrenia. *Am J Psychiatry* 2004; 161: 501–506.

- von Knorring AL, Cloninger CR, Bohman M, Sigvardsson S. An adoption study of depressive disorders and substance abuse. *Arch Gen Psychiatry* 1983; 40: 943–950.
- Wang XL, Mahaney MC. Genotype-specific effects of smoking on risk of CHD. *Lancet* 2001; 358: 87–88.
- Waterland RA, Jirtle RL. Transposable elements: Targets for early nutritional effects on epigenetic gene regulation. *Molecular and Cellular Biology* 2003; 23:5293–5300.
- Weaver ICG, Cervoni N, Champagne FA *et al.* Epigenetic programming by maternal behaviour. *Nature Neuroscience* 2004; 7: 847–854.
- Wernicke C, Smolka M, Gallinat J, Winterer G, Schmidt LG, Rommelspacher H. Evidence for the importance of the human dopamine transporter gene for withdrawal symptomatology of alcoholics in a German population. *Neurosci Lett* 2002; 333: 45–48.
- Whitfield JB, Nightingale BN, Bucholz KK, Madden PA, Heath AC, Martin NG. ADH genotypes and alcohol use and dependence in Europeans. *Alcohol Clin Exp Res* 1998; 22: 1463–1469.
- Wjst M. Target SNP selection in complex disease association studies. *BMC Bioinformatics* 2004; 5: 92.
- Wong DT, Reid LR, Li TK, Lumeng L. Greater abundance of serotonin1A receptor in some brain areas of alcohol-preferring (P) rats compared to nonpreferring (NP) rats. *Pharmacol Biochem Behav* 1993; 46: 173–177.
- Wong MY, Day NE, Luan JA, Chan KP, Wareham NJ. The detection of gene-environment interaction for continuous traits: should we deal with measurement error by bigger studies or better measurement? *Int J Epidemiol* 2003; 32: 51–57.
- Worst TJ, Vrana KE. Alcohol and gene expression in the central nervous system. *Alcohol Alcohol* 2005; 40: 63–75.
- Yates WR, Cadoret RJ, Troughton E, Stewart MA. An adoption study of DSM-III-R alcohol and drug dependence severity. *Drug Alcohol Depend* 1996; 41: 9–15.
- Yin SJ, Agarwal DP. Functional polymorphism of ADH and ALDH. In: Agarwal DP, Seitz HK (eds). *Alcohol in health and disease*. New York: Marcel Dekker, 2001: 1–26.
- Yoshida A, Ikawa M, Hsu LC, Tani K. Molecular abnormality and cDNA cloning of human aldehyde dehydrogenases. *Alcohol* 1985; 2: 103–106.
- Yoshida A. Molecular genetics of human aldehyde dehydrogenase. *Pharmacogenetics* 1992; 2: 139–147.
- Zhou FC, Pu CF, Murphy J, Lumeng L, Li TK. Serotonergic neurons in the alcohol preferring rats. *Alcohol* 1994; 11: 397–403.