# From genes to therapy:

## A study on how genetics can help identifying novel targets for alcohol dependence

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# Abstract

Alcohol addiction is a major public health burden. Treatment strategies available for alcoholism are limited in number and efficacy. This current review is focused on how genetic studies can contribute and have contributed to finding novel workable therapies for alcoholism. I address five candidate genes with strong evidence for associations with alcohol dependence: two ethanol-metabolizing genes (alcohol dehydrogenase enzyme, ADH1B, and aldehyde dehydrogenase enzyme ALDH2), one opioid receptor gene (OPRM1), one dopamine receptor gene (DRD2), and one GABA receptor gene (GABRA2). These genes show strong associations with alcohol dependence. Some functional polymorphisms predispose an individual for developing alcohol dependence, like those in the DRD2 receptor. In contrast, variants of the ADH1B and the ALDH2 genes are believed to have a protective effect, since the ethanol-metabolizing process is altered such that accumulated acetaldehyde leads to strong intoxication responses shortly after drinking alcohol (alcohol-induced flushing trait).

One candidate gene displays qualities that allow patient-tailored treatment based on their individual's genotype. The Asp40 variant allele of the OPRM1 gene improves the receptor ability such that treatment with naltrexone is more efficient, thus giving a subgroup of alcoholics a better chance of withstanding relapse. Despite discovering haplotypes on the DRD2 and GABR2 genes associating the dopaminergic and GABAergic system to alcoholism, drug treatments almost always lead to side-effects because of the complexity of these pathways.

Further research is required. I feel that genetic studies can contribute profoundly to developing drug treatments. With pharmacogenetic studies, it may be possible in the future to develop personalized drug treatments for alcoholism by making use of information on the genotype and the genetic predisposition (the contribution of genetic versus environment) related to the expected response to drug treatment.

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# 1 Heritability of alcohol addiction

## 1.1 Aim thesis

With increasing knowledge on genetics and the genetic make-up of individuals, scientists more and more try to dissect the exact genetic underpinnings of multiple diseases. This can give valuable information on the predisposition for a certain disease and the (neuro)biological mechanisms underlying diseases. Moreover, this may result in the development of novel treatment strategies for diseases that are difficult to treat pharmacologically until now. This applies to alcoholism also. Variation in the risk for alcoholism is defined for 40% to 60% by genetic variants (Schuckit, 1994). However, associating alcoholism with alleles, let alone, specific genes proves to be challenging. Nonetheless, genetic risk factors have been identified and thoroughly examined. This current thesis is aimed at evaluating the contribution of genetics to the development of new therapies; that is, the way genetic studies can contribute and have contributed to developing novel workable therapies for alcoholism. Also addressed is whether personalized therapies for alcohol addiction based on the genetic make-up of individuals are feasible. It is not the objective here to reflect on all genetic studies related to alcoholism, since that would go beyond the main underlying purpose of this thesis. The thesis will start off giving an overview on what alcohol addiction is, which factors contribute to the development of alcoholism, and how heritability of the disease is determined. The second chapter is on functional variances between people that provide insight in alcohol-response differences, the prevalence for developing alcohol dependence and the treatment this disease. It gives an example on how genetics have improved our knowledge on alcohol response and drug treatment. This thesis will end with a critical recapitulation on where in the field of genetics possibilities of improving the current therapies can be found.

## 1.2 Number and facts on alcohol misuse

Alcohol misuse is an acute health problem throughout the world. The vast majority of the world population – estimated at 2 billion people (WHO, 2004) - consumes alcoholic beverages. The World Health Organization (WHO) estimates that about 76.3 million people in the world suffer from diagnosable alcohol use disorders (WHO, 2004). (The exact definition of alcohol use disorders is described in the following section). In 2000, alcohol caused an estimated 1.8 million deaths (3.2% of total users), stressing the seriousness of the problem. In that same year, 4.0% of the global disease burden was related to alcohol, causing neuropsychiatric conditions and unintentional injuries (WHO 2004).

## 1.3 Alcohol abuse and alcohol dependence

Alcoholism is a psychiatric disease in which consumption of alcohol is sustained despite health problems and negative social consequences (Cloninger, 1987). Among other diagnostic methods, the Diagnostic and Statistical Manual of Mental Disorders, 4$^{th}$ edition (DSM IV) offers a checklist of diagnostic symptoms for many clinical addictions, including alcohol abuse and alcohol dependence – general medical terms for alcoholism (American Psychiatric Association, 1994). Differentiation is made between alcohol abuse and alcohol dependence.

### 1.3.1 Alcohol abuse

In general, alcoholism is measured by the maladaptive pattern of alcohol misuse leading to clinically significant impairment or distress (American Psychiatric Association, 1994). More specifically, alcohol abuse is manifested by 1) failures in fulfilling major obligations in life (work, school, and home); 2) recurrent alcohol use causing alcohol-related legal problems, 3) alcohol use leading to physically hazardous situations, and 4) continued alcohol use despite persistent or recurrent social or interpersonal problems. If one or more criteria are met within a 12-month period, an individual is considered to suffer from alcohol abuse.

### 1.3.2 Alcohol dependence

Alcohol dependence is set apart from alcohol abuse in that it involves also physical effects of alcohol (American Psychiatric Association, 1994). A compelling difference from alcohol abuse is the inclusion of criteria for tolerance and withdrawal. Of the following criteria, three or more have to be met within a 12-month period for the diagnosis alcohol dependence:

1. *Tolerance* – defined by: either, requiring higher consumption of alcohol to gain the same intoxicating effects, or, markedly diminished effect with continued use of the same amount of alcohol.
2. *Withdrawal* –defined by: taking alcohol to relieve or avoid symptoms caused by abstinence of alcohol (symptoms of withdrawal are autonomic hyperactivity, hand tremors, insomnia, nausea or vomiting).
3. Drinking larger amounts and over a longer period than was intended.
4. Persistent desire or unsuccessful efforts to cut down or control alcohol use.
5. Increasing time is spent on obtaining alcohol or recovering from its effects.
6. Given up or reduced important social, recreational or occupational activities in favor of drinking, and
7. Unable to give up alcohol use despite the knowledge of the destructive effects of alcohol on physics and psychology.

Overall, these criteria portray the elaborate effects of alcohol on not only social aspects, but also the neurobiology of the brain as shown by the first and second criteria, referring to tolerance and withdrawal, respectively. Moreover, the fourth criterion refers to the increased risk of relapse after chronic alcohol misuse. Relapse is an apparent chronic attribute of the disorder and it has to be kept in mind that, even though treatments of alcoholism are available, patients often show recurrent relapse periods (Ducci and Goldman, 2008). Alcohol has the potential of changing the biological systems in the brain potentially resulting in an alcohol-craving brain (Weinstein et al., 1998). As a consequence, individuals may display alcohol-seeking behavior in order to meet increased needs or to avoid withdrawal symptoms.

Important to note is that different types of alcoholics likely exist, which aspect of addiction is not dealt with in the current DSM-IV. Cloninger and colleagues have postulated the division of alcoholics into subgroups based on their behavioral characteristics. Personality traits such as impulsivity, risk-taking, emotionally dependence, perfectionistic and introvert were used to divide alcoholics into two main subgroups (Cloninger, 1987). The first group contains individuals with a passive-dependent personality that feel guilt and fear about their alcohol dependence with

characteristics leaning toward the latter three personality traits, referred to as the 'loss-of-control' type (type 1). The alcohol-seeking behavior (type 2) is associated with impulsivity, risk-taking and the tendency to antisocial behavior; with being inable to abstain entirely as the core-symptoms. Many alcohol abusers share features of both types, however, these alcohol-related syndromes reflect the polar extremes in personality. In contrast, diagnoses conform the DSM-IV checklist hold that, although both disorders, alcohol abuse and dependence, refer to an every day, maladaptive relationship with alcohol, the diagnose of alcohol abuse is rejected if within a 12 month period after diagnoses symptoms are measured that fall within the criteria of alcohol dependence; and the other way around.

## 1.4 Complex disorder

Despite the fact that development of addiction requires the initiation of ingestion of alcohol (Caetano and Cunradi, 2002), the use of alcohol does not necessarily predict the development of alcohol dependence. World-wide many people consume alcohol and only a relatively small percentage of those individuals become dependent on alcohol. This implies that other factors besides consumption play an important role in developing this type of disorder. Rainer Spanagel (2009) offered a definition which points out the complexity of the disorder, describing alcoholism as "a result of cumulative responses to alcohol exposure, the genetic make-up of an individual, and the environmental perturbations over time". The combination of genetic and environmental factors and the gene-environment interaction makes this topic complex to study.

## 1.5 Determining heritability

Extensive family, twin and adoption studies have documented a strong familial transmission and the underlying genetic mechanisms of alcoholism (Grant et al., 2009; Cloninger, 1987; Treutlein et al., 2009; Caetano and Cunradi, 2002; Ducci and Goldman, 2008; Reich et al., 1998; Foroud et al., 2000). Large sample studies have demonstrated a moderate but consistent contribution of genes to alcoholism, accounting for 40-60% of the risk (Kendler et al., 2003; Schuckit, 1994). An important question is which genetic differences explain inter-individual variation in the risk for alcohol dependence, e.g. the genetic variances associated with alcohol dependence. To determine the familial transmission, and thereby the vulnerability of individuals, samples are constructed out of unrelated individuals, i.e. healthy versus control, or related individuals, i.e. multigenerational families**.**

Linkage studies aim to identify chromosome regions that are shared more often among phenotypically concordant family members compared to phenotypically discordant relatives (Ducci and Goldman, 2008). Genetic linkage of twins or siblings samples provide more specific information about the distribution of variances related to alcohol dependence, where patterns of correlations in monozygotic (MZ) and dizygotic (DZ) twins are the most informative (Goldman et al., 2005). Whole genome association studies (WGAS) or genome-wide association studies (GWAS) analyze the whole genome of individuals, hereby comparing between large samples consisting out of subjects suffering from alcohol dependence and healthy controls. GWAS are characterized by their hypothesis-free nature. In contrast, candidate gene studies focus on specific genetic variances and their involvement in for example alcohol dependence.

### 1.5.1 Family studies

Family studies define the familial or genetic relationships of a disorder. Specific vulnerability factors will have genetic underpinnings, if a disorder is more common in relatives than controls. Family studies mostly use samples of multiple generations; starting of by selecting probands. A proband is the first affected person in a family that seeks medical attention for a disorder like alcoholism. Especially, with alcohol dependence being highly heritable, diagnosis of a proband raises levels of suspicion for other relatives (Reich et al., 1998). After setting the diagnoses, a pedigree chart can be drawn where affected but undiagnosed ancestors can be traced back. This approach of heritability research may suggest candidate genes if these family studies are followed by genetic linkage and association studies.

To give an example on family studies, the following multigenerational family study, conducted by Reich and colleagues looked for heritable vulnerability for alcohol dependence. They based their sample selection on probands diagnosed with alcohol dependence and their relatives. In this study, 23 females and 82 male probands with 3 or more affected first-degree relatives were selected depicting 987 informative individuals from 105 multigenerational families. This research revealed a 2 to 8-fold higher risk for siblings of alcoholic probands in comparison to controls. Risk ratios range from ~2.8% for brothers of males to 3.1% for brothers of sisters, and, 5.3% for sisters of male and 7.8% for sisters of females. These relative risks for alcohol dependence are sufficient to allow detection of genes of moderate effect size via a linkage study on genes of the affected sibling pairs. Strongest evidence for linkage loci to AD was found on chromosomes 1 and 7. One marker region on chromosome 2 pointed out increased sharing among affected sib-pairs. A later study confirmed the scores for chromosome 1 and 7 (Foroud et al., 2000). Linkage of chromosome 3 to AD was also observed. A remarkable different result was that, after combined analyses,

the marker regions on chromosome 2 were less obvious. In contrast to these findings stand a recent genome-wide association study (GWAS), which indicate the involvement of at least two markers on chromosome 2 in alcohol dependence (Treutlein et al., 2009). In this set-up, 1024 patients with alcohol dependence and 1358 control subjects are genotyped by extracting genomic DNA from whole blood samples (only males are included to increase the homogeneity of the sample). Single nucleotide polymorphisms, SNPs, served as markers. For the follow-up study, findings from gene-expression data in alcohol-dependent rats were integrated with findings of the GWAS. Of the in total 139 SNPs identified in the GWAS, 22 SNPs are located in human homologues of rat genes showing differential expression in the rat brain after long-term alcohol consumption. After genome-wide correction for multiple testing in a combined sample, two SNPs remained significant. These two markers are located 5kb apart in the chromosomal region 2q35, mapping to the (3'-flanking region of the) gene encoding peroxisomal trans-2-enoyl-coA reductase (PECR); this gene was associated with alcohol dependence in previous linkage studies. For conducting GWAS, inclusion of findings from animal studies will increase the power of the results. Moreover, this will provide additional information on possible SNPs that would otherwise have been excluded. For instance, the SNP located on the alcohol dehydrogenase (ADH) gene did not withstand correction for multiple testing in the GWAS and would have been excluded from the follow-up study if it had not been supported by evidence from the integrated animal study. The involvement of the ADH1B gene is described in more detail in chapter 2.

### 1.5.2 Twin studies

Among family studies, twin studies probably best show the heritability of alcoholism (Goldman et al., 2005). For clarifying the complexity of the transmission, differentiation has to be made between monozygotic and dizygotic twins. Two possible models of transmission are heterogeneity and polygenicity, both having an effect on the MZ/DZ twin concordance rate, which reflects the similarity of alleles for a pair of twins. Heterogeneity, the left part of figure 1, holds one transmitted risk allele that can be traced back in both monozygotic twins, but does not have to be traced back in both dizygotic twins. In this case, one single allele is determining vulnerability for alcohol dependence. Opposite to that, polygenicity refers to the involvement of multiple alleles that by simultaneous inheritance increase the risk for alcohol dependence, see right part of figure 1. Here, inheriting one allele does not increase the vulnerability, in contrast to inheriting the cluster of risk alleles, which will make individuals more susceptive for developing genetic disorders.

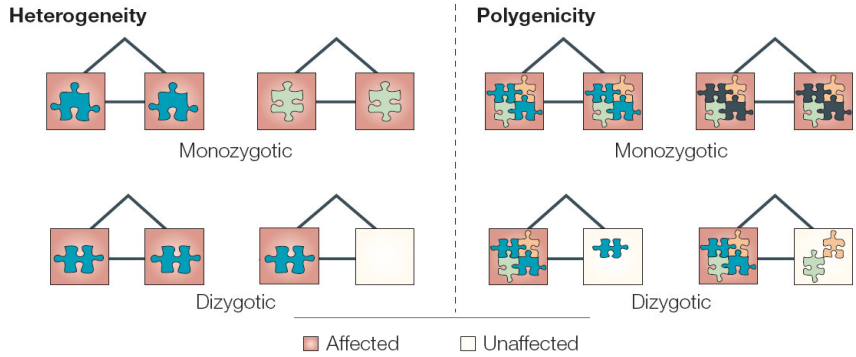


Figure 1: The genetic complexity in twin pairs. Every puzzle piece represents a risk allele. Figure derived from Goldman et al., 2005

Twin studies of alcohol dependence generally give a higher concordance among MZ twins then DZ twins, which is consistent with individual effects of alleles, i.e. a heterogeneous effect (as reviewed in Goldman et al., 2005). For instance, a study on alcohol dependence –with the primary goal of uncovering comorbidity – stated that life time prevalence for alcohol dependence (DSM IV) is around 23.9% in twin men (sample size: 3529 of which 866 MZ, 651 DZ, 510 unpaired twins) and 8.2% in women (sample size: 1929 women of which 866 MZ, 651 DZ, 510 unpaired twins) (Kendler et al., 2003). In comparison, a study on alcohol dependence in first-degree relatives of diagnosed alcohol addicts gave a prevalence of 37.1% in men and 20.7% in women (Nurnberger et al., 2004). A strong correlation between alcohol dependence and alcohol consumption is found in Australian twins and their spouses; genes contributing for 30%-51% to the extent of heavy alcohol consumption (Grant et al., 2009).

### 1.5.3 Adoptive studies

Family studies are by definition characterized by shared environmental factors and it is likely that gene-environmental interactions contribute to alcoholism also (Nurnberger et al., 2004). The most objective means of studying the gene-environment interaction for alcohol dependence - and diseases in general - is to study adoptees, i.e. the rates of alcohol dependence in adopted-away children with addicted biological parents compared to rates found in adoptees of parents with biological parents without alcohol dependence. Early research revealed that adopted-away sons of alcoholic biological parents had an 18% rate of alcohol dependence in comparison to the 5% rate of adoptees of control subjects (Goodwin and Schulsinger 1973; Cloninger et al. 1987) showing strong evidence for the genetic predisposition for developing alcohol dependence. Adopted away children more likely than others develop alcohol dependence when their biological parents have a history of alcohol misuse.

## 1.6 Environmental factors

In the above, 40%-60% of alcohol dependence was attributed to genetics. Environmental factors therefore must explain the remaining 40%-60% of the vulnerability to the disease. Parenting, socio-cultural like family and peer influences, demographic factors and economic and availability factors contribute to individual differences in vulnerability to initiating use and vulnerability to shift from use to addiction (Goldman et al., 2005). So next to the genetic make-up of an individual, certain lifestyle choices and events give an increased vulnerability for alcoholism. Drug-related cues and stress are also important factors to consider (Clarke and Schumann, 2009). As reviewed by Clarke and Schumann (2009), several animal studies revealed a relationship between stressful events and alcohol consumption. Experiencing stress has dysregulatory effects on the hypothalamic-pituitary-adrenal (HPA) axis, leading to increased cortisol levels causing psychological responses of the body in order to adjust behavior and thereby facilitate coping with stress. One of those coping behaviors may be alcohol consumption. In addition to genetic vulnerability factors, alcoholism is initially driven by consumption of alcohol and (sustained) drinking may induce alcohol tolerance, which may eventually lead to dependence (Caetano and Cunradi, 2002). When dependence on alcohol is established this has a further positive influence on the amounts of consumption (Grant et al., 2009). Note that positive here means increased consumption, which of course has in general negative consequences for the individual. Abstinence in young adolescents appears to be a critical factor, because every year that the onset of alcohol use is delayed will diminish the likelihood of alcoholism in adulthood by 4-5% per year (Goldman et al., 2005). Also, prenatally stressed rats convey increased vulnerability to substance abuse later in life (review by Clarke and Schumann, 2009). When translated to humans, this would imply that alcohol drinking by children and young adolescents should be prevented as much as possible (preferably, by good parenting). Certainly, considering both studies in humans and rodents, younger brains appear more susceptible for alcohol-induced brain damage and cognitive impairments than older brains (brains from adults) (National Institute of Alcohol and Alcoholism, NIAAA).

## 1.7 Comorbidity

Other risk factors can also be found on a more psychological level. Often psychiatric disorders such as ADHD (attention deficit hyper-activity disorder), depression, ASPD (antisocial personality disorder), nicotine abuse, cocaine abuse, and OCD (obsessive compulsive disorder), including alcohol dependence itself exhibit comorbidity

(Nurnberger et al., 2004). That is, individuals with alcohol dependence potentially have an increased risk to develop other psychiatric disorders (and visa versa) or multiple disorders are present at the same time. This complicates diagnoses and treatment of alcohol dependence on one hand, although, these disorders could also be used as a (genetic) indicator for increased risk for alcoholism. A comorbidity-study with first-degree relatives of probands on twenty-one disorders revealed that many disorders seem to cluster in families with an alcohol dependent proband (Nurnberger et al., 2004). This indicates that genes related to the increased risk for alcohol dependence may subsequently increase vulnerability for developing other disorders. A smaller study, on seven disorders, revealed no sex differences between men and women in the pattern of comorbidity, although the prevalence of these disorders differed between sexes. This is in agreement with epidemiologic (Caetano and Cunradi, 2002) and family and twin studies (Reich et al., 1998) depicting that, at least for alcohol dependence, women have lower rates than men.

## 1.8 Conclusion

Alcoholism is a complex disorder. Multiple factors contribute to the vulnerability for this disorder. As we have seen genes, environment and gene-environment interactions determine the heritability of alcoholism. This alcoholism can be determined in several ways. Indications for gene involvement come from multigenerational family studies where the prevalence of alcoholism throughout generations is determined and is indicative of the extent of heritability of alcoholism. Also, studies on siblings, twins and adopted children together with their biological parents and adoptive parents provide important information concerning the genetic predisposition for alcohol dependence. Linkage studies have lead to the identification of specific susceptibility genes for alcoholism.

The following chapter goes deeper into two genes with ethanol-metabolizing characteristics and one risk gene on the opioid receptor gene –all having polymorphisms depicting an increased risk for alcohol dependence. As will become clear, genetic studies helped identifying these risk genes and differences in response between individuals to alcohol (first example) and the treatment for alcoholism (second example).

# 2 Risk genes related to alcohol dependence

## 2.1 Alcohol dehydrogenase and aldehyde dehydrogenase genes

As stated in the previous chapter, genetic variances are considered to be a risk factor for the development of alcohol addiction. In uncovering genetic variances, one could look for obvious phenotypic characteristics as indicators for a genetic variance. A clear example is the strong intoxicative effect of alcohol on people with an Asian background; comparing affected Asians with other racial populations clearly identified polymorphisms on genes involved in ethanol-metabolism, which have been related to flushing and the risk for alcoholism (Kim et al., 2008; Sun et al., 2002; Bosron and Li 1986).

### 2.1.2 The flushing trait

Alcohol-induced flushing is a behavioral trait that is characterized by redness of the face, accompanied by other strong phenotypic responses such as dysphoria, tachycardia, nausea, palpitation, headache, drowsiness, breathlessness and hypotension (Bosron and Li, 1986; Kim et al., 2008). Symptoms will arise shortly after drinking even small amounts of alcohol and are a sign of intoxicating effects. Considering that facial flushing is the most obvious phenotypic characteristic, the trait owes his name to it. Strong alcohol-induced intoxicative effects are believed to protect an individual from 'over-drinking', thus thereby decreasing the risk of developing alcohol dependence.

The trait is highly common among the Asian population, which is, subsequently, the most studied population regarding this trait. However, genetic variances related to the alcohol-induced flushing trait have been studied in other world population as well (among that, the Danish population, Finns, and native Americans (Bosron and Li, 1986; Crabb et al., 2004; Tolstrup et al. 2008, Zintzaras et al., 2006). What makes this phenotypic trait a good study-objective is the strong genetic background. As will become clear, certain genetic variances are discovered that can be directly related to differences in alcohol-responses between subjects and controls. Before going deeper into the topic of genetic variances, it is necessary to first describe genes involved in the degradation process of alcohol.

### 2.1.2 Ethanol-metabolism

Together with the aldehyde dehydrogenase enzyme (ALDH; aldehyde: $NAD^+$ oxidoreductase; E.C. 1.2.1.3), the alcohol dehydrogenase enzyme (ADH; alcohol; $NAD^+$ oxidoreductase; E.C. 1.1.1.1) plays an important role in the oxidation ethanol (EtOH) (Stamatoyannopoulos et al., 1975). Ethanol oxidation, or degradation, occurs predominantly in the liver (vertebrates). This hepatic process starts by the degradation of ethanol to acetaldehyde catalyzed by ADH in the cytosol, whereafter mitochondrial ALDH converts acetaldehyde to acetate, see figure 1. Upon alcohol consumption ADH and ALDH genes are transcribed and enzymes are produced 'on demand' (Bosron and Li 1986). Polymorphisms on these genes are found to alter ethanol-metabolism. If alterations that change the enzymatic activity lead to accumulation of acetaldehyde, this will be accompanied by higher alcohol-induced intoxications (Stamatoyannopoulos et al., 1975; Crabb et al., 1989; Ducci and Goldman, 2008).

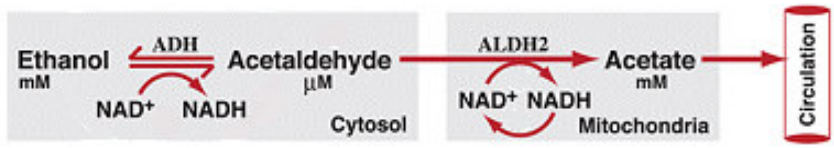


Figure 2: Scheme of the ethanol metabolism in vertebrates. The conversion of ethanol to acetate is catalyzed by the alcohol dehydrogenase (ADH) enzyme in the cytosol and the aldehyde dehydrogenase (ALDH2) enzyme in the mitochondria.

*Research in Drosophila melanogaster*

*Drosophila melanogaster* is one of the early-used animal models for genetic analysis, and are therefore also one of the genetically best-known organisms today. Years and years of experimenting with *Drosophila melanogaster* has provided a solid genetic basis to the understanding of underlying biological systems and genetic processes in humans and other eukaryotes, including the process of ethanol-degradation. Ethanol tolerance is a prevalent genetic adaptation of the *Drosophila* species to its own habitat and diet, where alcohol is abundantly present (Leal and Barbancho, 1992). Early electrophoresis and gel staining experiments in *Drosophila melanogaster* pointed out different alleles of the ADH gene ($ADH^{F(ast)}$ and $ADH^{S(low)}$) with different enzymatic activity (Grell et al., 1965). These kind of preliminary results led to many experiments on the influence of genetic variances on alcohol tolerance in fruit flies; being a convenient indicator for the internal alcohol-processes as death rate is a easy measurable parameter.

Wildtype flies (with active ADH) and mutants (with inactive ADH) in alcohol tolerance experiments show that ethanol degradation in *Drosophila* larvae is for over 90% regulated by ADH enzymes (Geer et al. 1985). Thus, ADH has a prominent role in the metabolism and detoxification of ethanol. Involvements of a second gene, the aldehyde dehydrogenase (ALDH), gained interest considering the following. The within-population variation in ADH can not fully explain the tolerance of natural species (Gibson et al. 1979). Experiments on ADH+ (active ADH) and ADH- (inactive ADH) flies showed that both were, respectively, very tolerant and relative tolerant for ethanol. This result was remarkable, considering that if ADH was responsible for the complete degradation process, flies with inactivated ADH should show high intolerance for ethanol (death following ethanol accumulation in body). Furthermore, it has to be considered that the metabolism of ethanol is a two-step process. Ethanol is degraded into acetaldehyde –a far more toxic product than ethanol (Leal and Barbancho, 1992). Acetaldehyde can be transformed back to ethanol via a reduction step catalyzed by ADH, but also further degraded to acetate via an oxidation process. In that final step, the enzyme aldehyde dehydrogenase (ALDH) plays an important role. Here, an example is given on how the involvement of both ADH and ALDH genes on alcohol tolerance is tested in *Drosophila melanogaster*. This experiment was executed by Leal and Barancho. Two ALDH activities can explain the in vivo transformation to acetate: from ALDH itself and ALDH from the ADH enzyme ($ALDH^{ADH}$). Tolerance was measured in an *in vivo* experiment by putting ADH+ and ADH- fruit flies on an ethanol or acetaldehyde medium and scoring death rate over time. ALDH activity in ADH+ and ADH- flies was inhibited by pre-feeding them with either cyanide (inhibits ALDH) or acetone (inhibits $ALDH^{ADH}$) and gas chromatography was used for *in vivo* determination of ethanol and acetaldehyde concentrations. As expected, high survival rates were measured in ADH+ flies on 0.5% acetaldehyde medium (survival rate of ~75% after 1hr), which is underlined by the relative low levels of ethanol and acetaldehyde in the bodies. Pre-feeding these flies with cyanide leads to high internal levels of both ethanol and acetaldehyde: Blockage of ALDH keeps acetaldehyde levels high, whereas a percentage is reduced back to ethanol (catalyzed by ADH). ADH- depict a lower tolerance for 0.5% acetaldehyde than the ADH+ (survival rate < 10% after 1hr) and pre-feeding ADH- flies with cyanide leads to elevated levels of acetaldehyde and ethanol. These levels are relatively lower than those in the ADH+, which is explained by the flies giving up feeding. Results show that in adult *Drosophila melanogaster*, the activity of ALDH is a prominent limiting factor in ethanol-tolerance in *Drosophila melanogaster* and that acetaldehyde detoxification asks for involvement of both ADH and ALDH enzymes.

Genetic determinations of the genome of the *Drosophila melanogaster* proved to be a good predictor for similar associations of genetic variances and alcohol tolerance in humans. As in the *Drosophila melanogaster* (Grell et al., 1965), polymorphisms on the human genes could result in different subunits (forming enzymes) with alternated ethanol-metabolism properties which could eventually lead to phenotypic differences in alcohol response. Criterion is that these polymorphisms, or mutations, occur on the active regions, i.e. exons, of the gene for it to be traced back in the phenotype. Mutations on both alleles will make an individual homozygous for that particular trait in comparison to heterozygous individuals who have mutations on only one allele. When there is no obvious dominancy of a certain allele with polymorphism, the corresponding phenotype is called an intermediate phenotype.

### 2.1.3 Polymorphisms on ethanol-metabolism genes in humans

Because of the alcohol-induced response (the flushing) and the possible connection to the process of alcohol metabolism, candidate gene studies where conducted for genes encoding for ethanol-metabolizing enzymes. Two functional loci on the ADH1B and the ALDH2 genes are addressed as most important (Bosron and Li 1986; Luczak et al., 2006; Ducci and Goldman, 2008). Polymorphisms, or more specifically single nucleotide point (SNPs) mutations, on these genes has lead to marked variances accounting for individual differences, i.e. the His47Arg polymorphism on the ADH1B gene and the Glu487Lys polymorphism on the *ALDH2* gene (Ducci and Goldman, 2008). The ALDH2 enzyme is encoded by the *ALDH2* gene located on chromosome 12 (human), and is involved in the conversion of acetaldehyde to acetate (figure 2). The defective or "atypical" *ALDH2* (Lys487) refers to a mitochondrial ALDH2-subunit that, after a single point mutation on position 487 (instead of glutamate now lysine is produced), shows a reduced activity compared to the common variant (Crabb et al., 1989). As a result, this inactivation of the enzyme prohibits the breakdown of acetaldehyde. Additionally, the Lys487 mutation appears to be dominant, thus either being homozygote or heterozygote will both predict a profound rise in acetaldehyde blood levels (Crabb et al., 1989).

Opposite to that, the His47Arg SNP on the ADH1B gene results in an increase in enzyme activity (Ikuta et al., 1988; Bosron and Li, 1986). The His47 allele produces a β2 subunit which is catalytically far more active than the Arg47 product, the β1 subunit. Being two to three times more efficient in ethanol elimination, possession of the (mutant) His47 variant potentially leads to higher acetaldehyde concentrations. A study among a Korean population showed a dramatic genetic effect on the risk of alcoholism when the two genetic effects are combined (Kim et al., 2008). Koreans with the usual genotypes (ADH1B* 487Arg and ALDH2*47Glu) have a 91 times greater risk for alcoholism than other who have only one polymorphism or

posses the atypical variances (ADH1B*487His and ALDH2*47Lys) (Kim et al., 2008). The first case of a chronic alcohol-dependent patient homozygous for both atypical genes (atypical *ADH1B* and atypical *ALDH2*) showed highly elevated blood acetaldehyde levels, increased heart rate and cardiac output, and facial flushing following ethanol intake (Chen et al. 1999).

### 2.1.4 Prevalence of polymorphisms in the World population

Alcohol-induced flushing is also often named the Oriental flushing trait; referring to the predominant occurrence of this SNPs on ethanol-metabolizing genes in Asians. It was this striking intoxicative response among Asians that hinted in the direction of a possible genetic association with a phenotypic response to alcohol. By assaying hair root or liver samples, these studies showed that about half of Orientals are heterozygote or homozygote for the Lys487 allele and thus, consequently, lack ALDH2 activity (Goedde 1980, Harada 1980). This is much more in comparison to European populations. Moreover, population studies showed that Asians predominantly carry the "atypical" His47 variant of the ADH1B gene (~85% allele frequency), whereas the Caucasians genotype predominantly presents the "usual" Arg47 variant (>85% allele frequency) (Ikuta et al., 1988; Stamatoyannopoulos et al., 1975; Park et al., 2006; Bosron and Li 1986). Numerous population studies pointed out racial differences in both the ADH and ALDH genotype. Koreans (Kim et al., 2008), Japanese (Sun et al., 2002), Chinese and Thais (Luczak et al., 2006) mostly possess the atypical polymorphism. In contrast, the usual variations are predominantly found in European Caucasians (Bosron and Li 1986), including the Danish population (Tolstrup et al., 2008), and, in American Caucasians and African Americans (Bosron and Li 1986). Furthermore, studies are conducted in far more world populations; all more or less pointing out the protective effects of the atypical genotypes of ADH1B and ALDH2.

### 2.1.5 Acetaldehyde levels in the blood

Considering all this, possession of the mutant alleles of the ADH1B (487His) and the ALDH2 (47Lys) gene is believed to give out a positive and even protective effect against the development of alcohol tolerance and dependence; assuming that acetaldehyde accumulation is very important in withholding individuals from drinking excessive amounts of alcohol (Quertemont et al., 2009). The hypothesis needs to be tested whether polymorphisms on the ethanol-metabolizing genes depict higher levels of acetaldehyde which subsequently decreases ethanol-intake; thus, whether these polymorphisms are functional. A recent elegant study demonstrated nicely that elevated levels of acetaldehyde, as a consequence of polymorphism on the ADH1B gene, have a protective effect against alcoholism. Alcohol-preferring rats were injected with mutant cDNA mimicking the "atypical" His47 variant of the ADH1B gene

in humans (for details, see Rivera-Meza et al. 2009). After ethanol administration, 3 to 5-fold higher acetaldehyde blood concentrations were found in the *rADH-47His* rats than those in animals transduced with the wild-type *rADH-47Arg*. Next to that, these rats showed a 50% reduced ethanol-intake and results are supported by many other studies (e.g., Quintanilla et al., 2007). Possession of the mutant ALDH2 allele also decreases ethanol consumption as seen in ethanol-free-fed rats that where pretreated with an anti-*ALDH2* antisense gene (inhibiting ALDH activity) (Ocaranza et al. 2008). A drug therapy of this kind is disulfiram, an ALDH antagonist described in the next part.

### 2.1.6 Disulfiram

Following the discovery of polymorphisms on ethanol-metabolizing inhibiting ALDH enzyme activity, it is better explained why alcohol-sensitizing drugs such as disulfiram help decrease the risk of relapse. Disulfiram is a known aldehyde ALDH inhibitor (Quertemont et al., 2005). It blocks ALDH activity by forming intramolecular disulfide bridges (Shen et al. 2000), resulting in acetaldehyde accumulation depicting similar physical responses as seen in Asians with genetic protection against excessive alcohol consumption. In fact, the response to alcohol preceded by disulfiram administration is called the disulfiram-ethanol reaction. Disulfiram has been used clinically for over 50 years in the treatment of alcoholism. After all, the same pharmacological responses (aversive reaction upon alcohol consumption) are met and these drugs have a strong psychological deterrence-effect (decreasing the motivation to start drinking and experiencing the unpleasant consequences of alcohol).

*Limitations to disulfiram*

There are several limitations to the use of the drug (Brien and Loomis 1985). Firstly, disulfiram is not highly selective for only hepatic ALDH, but also inhibits at least three other enzymes. Among them, the dopamine β-hydroxylase – a stress hormone and neurotransmitter that converts dopamine to norepinephrine. Secondly, its onset of approximately 12 hr is a disadvantage since immediate protection against alcohol consumption is preferred (when starting off with the treatment). On the other hand, the inhibition of the ALDH enzyme will last 6 to 10 days (Brien and Loomis 1985) giving the benefit of not requiring disulfiram ingestion daily over the course of rehabilitation. The irreversibility of the inhibition is the fourth limitation of disulfiram. Blocking hepatic ALHD will lead to increased levels of acetaldehyde in blood and liver (Eriksson 1985) and continued high levels of acetaldehyde is toxic to the body, resulting in liver damage and toxicity to the cardiovascular and CNS system. Furthermore, elevated levels of acetaldehyde can cause displacement if inhibitor-

affinity is not high (Feldman et al., 1996). Side-effects of disulfiram are drowsiness, lethargy, tremor, dizziness, and headache (Feldman et al., 1996). Another disadvantage on a human level has to do with the voluntary intake of the drug by patients. Patient compliance is important, but this is stretched when patients with often psychological or psychiatric problems are asked to take a drug that will make them heavily dislike their most favorite beverage.

#### 2.1.7 Conclusion

Alcohol-induced flushing is a behavioral symptom with a clear genetic component. The heritability and related phenotypic features makes the alcohol-induced flushing a good study topic for genetic variances. Early studies on the phenotype of *Drosophila melanogaster* have indicated that genetic variances in alcohol-metabolizing genes account for differences in alcohol tolerance. Relating this to human studies, defective ethanol-metabolizing genes, the alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), will lead to differences in alcohol-vulnerability between individuals. Research on the ADH and ALDH genes contributed to our knowledge on how genetic differences account for differences between individuals in developing alcohol dependence. In conclusion, the genetic protective effect of the atypical (mutated) genotypes prevents an individual, namely the Asians, from heavy drinking; thus diminishing the susceptibility for alcoholism.

## 2.2 The opioid receptor gene

Several drug treatments are available to prevent patients from relapsing after alcohol withdrawal, although the results are contradictory. Here the μ-opioid receptor (OPRM1) gene will be discussed as an example of beneficial pharmacogenetic approaches. This gene is of particular interest because functional polymorphisms in this receptor gene have been related to the response to pharmacological treatment.

### 2.2.1 Ethanol and the opioid receptor gene

Animal studies preceded the involvement of the OPRM gene in showing a relationship between alcohol and the endogenous opioid system (Volpicelli et al., 1995). Alcohol consumption stimulates endogenous opioid activity (Gianoulakis, 2001). The sense of euphoria humans experience is thought to be caused by the release of opioid peptides (King et al., 1997). In case of chronic heavy drinking, a central opioid deficiency is induced leading to feelings of craving (Gianoulakis, 2001). Also, human studies suggested that experiencing stressful events is related to a sustained deficiency in the endogenous opioids afterwards (Volpicelli et al., 1995). This opioid deficiency will

cause patients to relapse; perceiving the deficiency as opioid withdrawal and thereby promoting alcohol consumption (Gianoulakis, 2001).

### 2.2.2 Naltrexone

Administering the μ-opioid receptor antagonist naltrexone proved to prevent the increase of endogenous opioids by alcohol and also blocks the euphoria produced by alcohol (King et al., 1997; Volpicelli et al. 1995). Furthermore, naltrexone is one of the FDA (US Food and Drug Administration) approved medication for patients rehabilitating of alcohol dependence (Weinrieb and O'Brien, 1997). Despite its positive influence on decreasing the risk for relapse in most studies, some studies failed to show significant drug-placebo difference (Kranzler 2000; Krystal 2001). It was suggested that the differences in drug response between studies and subjects could have its origin in genetic differences between subjects; clinical trails on alcoholism that took the family history in consideration pointed in that direction (Jaffe et al., 1996). Considering the efficacy of naltrexone –being an opioid receptor antagonist– pharmacogenetic research directed to the μ-opioid receptor gene (OPRM1) has been conducted.

In line with this hypothesis, indeed genetic differences between individuals were found on the OPRM1 gene. In particular, a functional single point mutation on position 118 (Asn40Asp) on exon 1 of the OPRM1 gene has been studied widely in relation to alcoholism. Instead of the protein asparagine (Asn; aminosequence AAU or AAC) now aspartic acid (Asp, amino sequence GUA or GAC) is implemented (Oslin et al., 2003). Consequences of this A118G polymorphism is altered receptor affinity for β-endorphin, the endogenous opioid that activates the OPRM1 receptor (Bond et al., 1998). Receptor binding of β-endorphin is 3-fold better in individuals with the Asp40 allele compared to Asn40 allele possessing individuals. Furthermore, people with the mutant allele show greater response to alcohol than controls (Ray and Hutchinson, 2004).

Oslin et al. (2003) were the first to show that individuals with a functional polymorphism on the OPRM1 gene responded significantly better to naltrexone-treatment in comparison to the placebo group. Possession of one or more Asp40 alleles proved beneficial with respect to the treatment, while both the naltrexone-group homozygote for Asn40 and the two placebo-groups homozygote or heterozygote for Asn40 allele scored significantly worse in the number of days of survival (time to relapse). These are strong indications for altered ethanol-response as a consequence of genetic mutations, whereby these same mutations have a different effect on the efficacy of the used drug-treatment.

### 2.2.3 Conclusion

Ethanol has an euphoric effect on the brain, which involves the endogenous opioid system that is enhanced following ethanol consumption. Deficiencies of this opioid system makes alcohol addicts more vulnerable to relapse (by experiencing feelings of cravings). As described here, naltrexone works better in some addicts than others in preventing relapse. Functional polymorphisms are found on the OPRM1 gene that first of all lead to differences in alcohol response – people with the Asp40 mutant have a higher receptor affinity for alcohol – and secondly, enhances the receptor affinity for naltrexone – thereby improving the working of the opioid receptor antagonist in preventing relapse. This particular example shows that a pharmacogenetic approach of alcoholism can be grounded: it indicates that there are subgroups of alcoholics to be considered, based on their OPRM1 mutation, that show differences in response to drug treatment as they already had differences in their initial response to alcohol. Furthermore, it demonstrates that it pays off to consider the genetic predisposition for alcohol dependence and that genetics can help determine the optimal treatment for alcoholism.

# 3 Other risk genes for alcohol dependence

## 3.1 Neurotransmitter systems affected by ethanol

Feelings of euphoria after ethanol consumption are associated with increased synaptic dopamine (DA) which is entwined with complex changes in numerous neurotransmitters, including GABA (γ-aminobutyric acid), glutamate, serotonin (5-HT), opioids peptides and cannabinoids (McBride, 2002). DA and 5-HT are, next to (nor)adrenalin, important neurotransmitters involved in reward and emotional processing. Besides the mesolimbic dopamine system, which is considered the brain reward system (Pierce and Kumaresan, 2006), the central nucleus of the amygdala (CeA), the 'emotion-center' in the brain, is implicated in regulating alcohol-drinking behavior (McBride et al., 2002). Microdialyses studies in rats and mice have shown that acute ethanol injections result in increased DA and 5-HT release in the nucleus accumbens and also the CeA. $GABA_A$ receptors within the CeA are involved in oral ethanol self-administration (McBride et al., 2002; Hyytia et al., 1995). Not surprisingly, several genes associated with alcoholism belong to these neurotransmitter systems. Two examples will be described here: the dopamine D2 receptor gene and the GABRA2 receptor gene.

## 3.2 Dopamine receptor gene

### 3.2.1 Ethanol and dopamine

Dopamine (DA) plays an important role in the motivational aspects of addiction (Kalsi et al., 2009). The mesolimbic dopamine system, often referred to as the 'reward pathway', is involved in alcohol reinforcement and the development of alcohol dependence (Franken et al., 2005). It is hypothesized that decreased levels of DA in the brain increase the risk for developing alcohol dependence, in that progressively more alcohol consumption is required to compensate for the decrease in activation of the associated reward circuits (Phillips et al. 1998). For instance, mice lacking dopamine D2 receptors (DRD2) show a reduction in alcohol consumption of 50% compared to wildtype mice (Philips et al. 1998). Reduced densities of dopamine D2 receptor were measured in the central nervous system (CNS) of alcohol-preferring rats (McBride et al., 2002), suggesting that alcohol consumption is related to dopamine concentrations. Overexpression of DRD2 receptor reduces alcohol self-administration (Thanos et al., 2005; Thanos et al., 2001): Microinjections with a vector (gene transfer via adenoviral vector) to overexpress the DRD2 in the nucleus

accumbens (NAc) lead to reduced alcohol preference and intake in both alcohol preferring rats and non-preferring rats. These findings suggest that high levels of DRD2 may be protective against the development of AD. At first glance, these findings seem contradictive to that of the reduction of ethanol drinking in DRD2-deficient mice. It is perhaps possible that the role of DRD2 in regulating drinking behavior differs between alcohol-preferring and non-preferring animals (suggested by Thanos et al. 2001), a factor that is not taken into account in the knock-out experiment.

Next to the effect of dopamine levels on ethanol consumption, ethanol, on the other hand, has also a direct dopaminergic effect. Following voluntary ethanol intake stimulation of DA synthesis was measured in the rats' nucleus accumbens (Nac) (Weis et al., 1993). Furthermore, a combined case-control and family study in humans showed that detoxified but previously heavy drinking men depict reduced DRD2 receptor function (Wiesbeck et al., 1995). Moreover, this reduced dopaminergic receptor function contains inheritable components, as significant differences are measured between receptor function of dependent subjects and controls when family history with alcohol dependence was taken into consideration. Consequently, when taking the aim of this thesis in account, the questions arises whether (1) functional polymorphisms in the D2 receptor exist and associate with alcoholism and (2) whether D2 receptors could be useful therapeutic targets for alcoholism, possibly in a genetically distinct subpopulation only. The next paragraph will describe the polymorphisms found to be functionally disrupting the DRD2 receptor.

### 3.2.2 Polymorphisms in the Dopamine D2 receptor gene

Studies on other drugs of abuse, such as nicotine and opium, have identified a polymorphism on the *DRD2* receptor gene to be associated with these addictions (Blum et al., 1990). Association studies in populations of alcohol abusing and alcohol dependent subjects have shown that higher frequencies of this DRD2 Taq A1 polymorphism are also associated with alcohol dependence (Connor et al., 2002; Noble, 1998; Blum et al. 1990), with low *DRD2* density (Thanos et al., 2001), and with an antisocial personality, which may pose an increased risk for developing Cloninger's type 2 alcoholism (Ponce et al., 2003). Possession of this Taq A1 allele increases mortality rates in unrelated alcohol dependent individuals, as was shown in a 10-year follow-up study (Berggren et al., 2009)**.** The Taq A1 polymorphism is actually located 10Kb downstream of the DRD2 gene, in the neighboring ankyrin repeat and kinase domain containing 1 –the *ANKK1* –gene, which was also associated with the disorder (review by Kalsi et al., 2009).

Earlier this year, conclusions of a recent study conducted by Kraschewski and colleagues on polymorphisms with a possible relation to alcoholism came out. The aim of this study was to investigate whether putative functional polymorphisms, particularly which specific haplotypes of the DRD2 gene, are associated with alcohol dependence. Haplotypes refer to combinations of alleles at multiple loci transmitted together on the same chromosome. In other words, receptor expression may be affected by several polymorphisms located on both alleles and transmitted as a cluster. Several polymorphisms were identified and related to Cloninger's type 1 and type 2 alcoholism, but better results were obtained by looking at haplotypes. Four haplotypes (I-T-A-A2, I-C-G-A2, I-C-A-A1, D-C-G-A2) accounted for more than 80% of the all haplotypes coding for DRD2. The case-control sample showed a higher frequency for haplotype I-C-G-A2 in patients compared to controls, and I-C-A-A1 was observed more frequently in the whole group of alcoholics. Both haplotypes have also been associated with reduced dopamine D2 receptor availability, which as we have seen, increases the vulnerability for alcohol dependence and can therefore both can be considered risk haplotypes.

### 3.2.3 Pharmacology and the DA receptor gene

In light of the above, treatments for alcohol dependence would be expected to benefit from dopamine D2 agonist, which would increase the receptor function, thereby enhancing dopamine levels and, subsequently, reducing alcohol consumption. Matters are more complex than this assumption, since the more selective dopamine agonist lisuride did not result in decreased risk for relapse or in longer time latencies in resumed drinking (Schmidt et al., 2002). In a double blind study, lisuride was given to detoxified alcoholics; receiving drug or placebo for 6 months followed by 6 months of monitoring without any drugs. Remarkable was that patients receiving lisuride but expecting placebo gave the highest relapse rate; within 8 months all relapsed. Of the subjects that expected lisurdine, 20% stayed abstinent after the year. The genetic make-up of the subjects was not taken in account, perhaps later experiments that do will give more insight in these unexpected results. Furthermore, although used for treating, among others, neurodegenerative diseases (like Parkinson's disease) and psychiatric disorders (like schizophrenia), dopamine (ant)agonists will influence a broad range of neurotransmitter system leading to several physical side-effects to consider (Rang et al. 2007). Hallucinations, psychosis, hypotension, nausea, insomnia, but also parkinsonism, hyperprolactinaemia, and tardive dyskinesia are some side-effects to take in account regarding pharmacological treatments.

## 3.3 $GABA_A$ receptor gene

### 3.3.1 Ethanol and $GABA_A$ receptor

GABA stands for γ-aminobutyric acid and has multiple functions in the central nervous system (CNS); GABAergic neurotransmitter modulate among others emotions and responses to stress (Purves et al. 2006; Kumar 2009). Moreover, these neurotransmitters are mostly found in local circuit interneurons, thereby exerting control over the DA pathway as well. Where glutamate – a GABA precursor – is an excitatory neurotransmitter, GABA is an inhibitory neurotransmitter (Enoch, 2008). Three types of postsynaptic receptors are employed by GABA: the $GABA_{A,}$ $GABA_B$, and $GABA_C$ receptors. I will focus here on the first one. $GABA_A$ receptors fall under the ionotropic receptors, a family of chloride ($Cl^-$) ion channels. The inhibitory characteristics of this receptor subtype are explained by the hyperpolarization of the membrane as a consequence of the influx of Cl- following GABA binding to the $GABA_A$ receptor.

Many behavioral effects of ethanol are believed to involve GABAergic mechanisms (Kumar et al., 2009). For instance, sedative-hypnotic effects and motor incoordination (including decreased muscle tone) following ethanol consumption may be caused by the activation of $GABA_A$ receptors and, consequently, increased inhibition of postsynaptic receptors, and blocking receptor activity leads to postsynaptic excitation, including anxiolytic, anticonsulvant and seizurestic behaviors (Kalsi et al., 2009). Several studies have examined the acute and chronic effects of ethanol on the $GABA_A$ receptors (Nowak et al., 1998; Buck and Harris, 1990b; Sarviharju et al., 2006; Weiner and Valenzuela, 2006; Ashok 2005; Enoch, 2008; Buck and Harris, 1990a). Nowak and colleagues blocked the $GABA_A$ receptors by injecting a $GABA_A$ receptor antagonist to examine the acute effects on ethanol consumption. Picrotoxin is, like biculline, a $GABA_A$ receptor antagonist which one would predict could reduce ethanol intake. Microinjection with picrotoxin in the anterior ventral tegmental area (VTA) in alcohol-preferring rats indeed attenuates ethanol intake of the alcohol-preferring (P) rats, thus suggesting involvement of $GABA_A$ receptors in alcohol-drinking behavior (Nowak et al., 1998). Microinjections with another $GABA_A$ inverse agonist (antagonistic action on $GABA_A$ receptor, SR95531) in the extended amygdala also reduced ethanol response in rats (for details, see Hyytiä and Koob, 1995). This is in line with the above described involvement of the $GABA_A$ receptors activity on the dopamine system. As a result of inhibitory effect of $GABA_A$ receptors on the postsynaptic dopamine receptors, dopamine levels will rise in the emotion related areas in the brain, like the extended amydala, thereby altering the ethanol intake.

### 3.3.2 Polymorphisms on the $GABA_A$ receptor gene

When studying $GABA_A$ receptors, the complexity of this neurotransmitter system is as interesting as overwhelming. GABA receptors consist of 5 subunits, which can be combined in multiple ways to form $GABA_A$ channels, which all contain α subunits. The most common type of $GABA_A$ receptors in the brain however is a pentamer comprising two α's, two β's, and a γ ($\alpha_2\beta_2\gamma$) (Ashok et al., 2005). These genes are divided as follows: six types of α subunits (GABRA1, GABRA2, GABRA3, GABRA4, GABRA5, GABRA6); three β's (GABRB1, GABRB2, GABRB3); four γ's (GABRG1, GABRG2, GABRG3, GABRG3); as well as a δ (GABRD); an ε (GABRE); a π (GABRP); and a θ (GABRQ). Researchers have worked hard on unraveling the role of each $GABA_A$ receptor gene in alcohol tolerance, withdrawal, dependence, and relapse, and the most compelling results are shown here.

The *GABRA2* gene, coding for the α2 subunit, carries several polymorphisms associated with alcohol dependence (Edenberg et al., 2004; Agrawal et al., 2006; Fehr et al., 2006). In linkage disequilibrium analyses of 69 SNPs within a cluster of four $GABA_A$ receptors genes association with alcoholism was tested (Edenberg et al. 2004). Linkage disequilibrium (LD) rests upon the assumption of polygenicity, referring to haploblocks or haplotypes when SNPs are transmitted in clusters. Different SNPs are perceived as a possible cluster (haploblock) associated with a disease or trait when LD is high. The International HapMap Consortium constructed a haplotype map of the complete human genome that can be used as a guidance for generalized research (International HapMap Consortium, 2005). Here, a single haploblock on intron 3 past the 3'end of the GABRA2 gene was found with a higher LD among alcoholics than controls; suggesting association of that particular haplotype with alcohol dependence (Edenberg et al. 2004). These findings were later confirmed by Fehr and colleagues (2006), but only partly by Agrawal et al (2006) who demonstrated that only subjects with comorbid illicit drug dependence showed association for GABRA2. Furthermore, evidence for increased alleles sharing (SNPs) was found in the *GABRB1* locus (Reich et al. 1998), but later invalidated (Edenberg et al., 2004). Association for alcohol dependence for $\alpha_1$ and $\alpha_6$ subunits, respectively encoded by *GABRA1* and *GABRA6*, was confirmed by a human association study in Koreans (Park et al. 2006) and an animal study on Sardinian alcohol nonpreferring and Sardinian alcohol preferring (Conguddu et al. 2003). Pharmacologically, the *GABRA2* gene received the most interest, being a target of benzodiazepines (Buck and Harris, 1990a and 1990b; Campo-Soria et al., 2006). Benzodiazepines have an enhancing effect on $GABA_A$ receptors, possible by changing receptor affinity for GABA, as seen with diazepam (Campo-Soria et al. 2006). Benzodiazepine and barbiturates,

drugs acting as a GABA agonist, are used to treat epilepsy and are effective sedatives and anesthetics (Purves et al., 2004).

### 3.3.3 Pharmacology and the *GABRA2* receptor gene

As outlined above, functional polymorphisms, even clustered SNPs, on the *GABRA2* gene have been associated with alcohol dependence. Research on the possession of a particular haplotype depicting differences between alcoholics in their response to pharmacology as seen with the OPRM1 polymorphism, is very scarce. Most studies are on acute and chronic effects of ethanol on receptor regulation and gene expression or on how gene expression can alternate the ethanol consumption. This kind of research is certainly valuable in the interpretation of the mechanism of ethanol in the brain. Despite the clear important role of $GABA_A$ receptors in the effects of alcohol and the susceptibility for alcohol dependence, unfortunately not many clinical studies on the genotype-dependent treatment effects are executed.

Having this said, knowledge on variances between individuals and differences in vulnerability of developing alcohol dependence has proven valuable information in pharmacological treatments. The next and final chapter will describe the possible benefits of the study field of pharmacogenetics.

# 4 Candidate genes and treatment of alcohol dependence

## 4.1 A recapitulation

The scientific world has been very successful at unraveling the human genome, although this does not yet explain the mechanism and function of all genes. After all, the exact genetic relation to many diseases and disorders remains elusive. Nonetheless, scientists just have made tremendous progress in incorporating genetic knowledge to the field of alcohol addiction in order to get a perception of the mechanism and to find improvement for the current drug treatments. The many studies directed at the interplay between the wide range of genes, neurotransmitter systems, and ethanol consumption has improved our understanding of the mechanisms of ethanol in the brain.

The goal of this thesis was to look for possible targets and approaches to identify new pharmacological treatments for alcoholism from genetic association studies. Many genes have some degree of association with alcohol dependence, as the below overview of all current studied genes in their relation to alcoholism shows (figure 3).

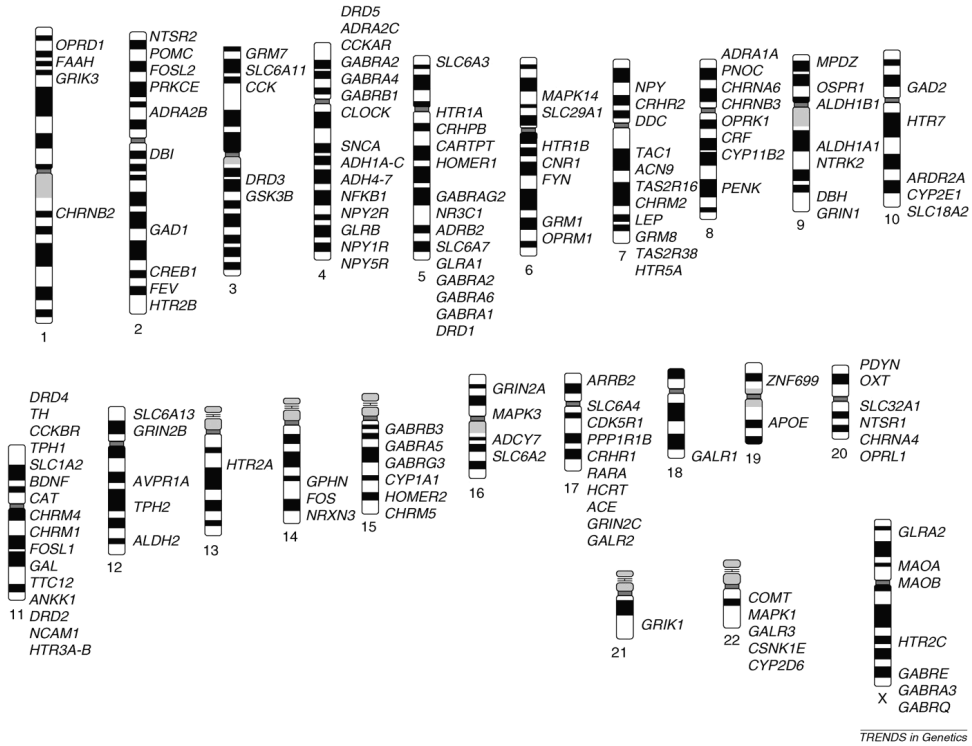


Figure 3: List of candidate genes involved in AD and alcohol-related phenotypes. Figure derived from Kalsi et al., 2009

The amount of current pharmacological treatments available stands in sheer contrast to this enormous list of candidate genes. Out of this list, five candidate were chosen to study, two ethanol-metabolizing genes (ADH1B and ALDH2), one opioid receptor gene (OPRM1), one dopamine receptor gene (DRD2), and one GABA receptor gene (GABRA2). These five genes have most consistently and frequently been associated with alcohol dependence. However, we do not know the exact mechanism through which most of these genes influence vulnerability for alcohol dependence, let alone how they are involved in addiction. The mechanism through which the ADH and ALDH variants may protect against alcoholism are probably the most clear. Namely, possession of a mutant ADH1B allele (His47) and ALDH2 allele (Lys 487) causes major differences in their acute, aversive response to alcohol as compared to individuals with the most common variant of these genes.

In respect to the general question of this thesis, the pharmacogenetics approach of alcoholism is best promoted by the OPRM1 gene. The value of pharmacogenetic studies is explained by this specific mutation, since OPRM1 is strongly associated with alcohol dependence, difference in alcohol response are measured between individuals with (Asp40) and without (Asn40) polymorphism on the alleles, and having the Asp40 allele makes drug treatment work more efficient in preventing relapse in comparison to having the Asn40 allele. **Why do these other candidate genes fail at showing the same results?** Well, it is not a failure as it is more a lack of knowledge. Studying one pathway on itself is quite impossible because of the complex connections with other neurotransmitter pathways. Add to that the characteristics of ethanol of not being selective and interplaying with multiple systems throughout the brain, and it becomes clear why every question answered brings up several new questions. On the bright side, there is evidence for the heritability of alcohol dependence and many candidate genes are found with potential functional polymorphisms altering the vulnerability for alcohol dependence. However, how can these candidate genes be further studied in their vulnerability-increasing potential? Haplotype studies are more informative than microarray studies were ~500.000 SNPs are studied at once (Kraschewski et al., 2009). Since variants must be very common to be associated with susceptible genes (Buckland 2008), clustering of SNPs will increase the statistical power (Kraschewski et al., 2009). On the other hand, this approach involves large samples and a negative aspect about large sample studies is the large clinical heterogeneity as multiple genetic and environmental factors contribute to alcoholism (Spanagel 2009). This asks for an individualized approach.

## 4.2 Tailor-made pharmacogenetic treatments for alcoholism –a dream?

Is it a dream to have tailor-made drug treatments for diseases that have a strong genetic background? What we have come to know is that alcohol dependence is genetically as well as environmently determined and that many polymorphisms on genes are identified that depict structural and functional alternations resulting in a changed response to alcohol. Perhaps we should redirect our focus and divert away from the complex system such as the dopaminergic and GABAergic system. Pharmacological compounds aimed at these systems easily yields unwanted side-effects. Still, keeping in mind their involvement in many behavioral effects related to ethanol consumption, these systems should not be overlooked. A good approach for studying the dopaminergic and GABAergic systems would be by focussing on relative less common but functional genetic variants nearby the current candidate genes and in hopes of finding new polymorphisms depicting functional changes in these systems. Another suggestion for finding effective new drugs is by identifying new putative compounds in preclinical models, such as genetically defective mice models, and translating this to small inpatient test trail (Spanagel 2009). Positive outcomes of the potential efficacy of these drugs will justify larger scale studies if association studies have indicated variants being common in at least some subgroups of alcohol addicts.

In conclusion, the complexity of alcohol dependence asks for a constant collaboration between scientist working in the fields of molecular biology, genetics, and (pre)clinical set-ups. The relative new field of pharmacogenetics is has the potential to aid in the development of personalized drug treatments for alcohol dependence as it relates the efficacy of pharmacological compounds to the genetic make-up of individuals (or at least, subgroups). Therefore, a strong recommendation for future research would also be to combine association studies with pharmacology studies.

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