

# Genetic Polymorphism in Alcohol-dependent Genes: A Review

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## ABSTRACT

Alcohol dependence (AD) is a complex multifactorial disorder that poses a serious medical and sociological problem. Neurobiology of drug abuse helps us understand the genetic, cellular, and molecular mechanisms that influence transition from occasional, controlled use to loss of control in drug-seeking behavior. Elements of impulsivity and compulsivity yield a composite three-stage addiction cycle mediated by discrete neurocircuits involving the basal ganglia, extended amygdala, and prefrontal cortex. Genetic polymorphisms of the genes encoding alcohol metabolism enzymes and neurotransmitter signaling molecules in dopamine (DA) and opioid systems substantially contribute to individual variations of susceptibility to AD. The primary enzymes involved in alcohol metabolism are alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). Genetic variants of these genes result in acetaldehyde accumulation and hence have a protective effect on the risk of alcoholism. Yet another mutant variant of microsomal enzyme cytochrome *P 450 2E1 c2/c2* that is found to be associated with higher transcriptional activity might play a role in the development of AD. Though functional variant 118G allele in exon1 of the  $\mu$ -opioid receptor (*OPRM1*) gene has been associated with the development of AD, few clinical studies do not unequivocally support the association. Dopamine is an important neurotransmitter involved in reward mechanism, and the most studied genetic variant of DA D2 receptor (*DRD2*) gene has been found to be associated with increased AD risk.

**Keywords:** Alcohol dependence, Alcohol metabolizing enzymes, Dopamine, Dopamine receptor, Genetic polymorphism, Opioid receptor.

*SBV Journal of Basic, Clinical and Applied Health Science* (2020): 10.5005/jp-journals-10082-02260

## INTRODUCTION

Alcohol use disorder (AUD) is a chronic relapsing brain disease characterized by an uncontrollable use of alcohol despite adverse social, occupational, or health consequences.<sup>1</sup> Nearly 5.1% of the global burden of disease is attributable to alcohol consumption, and it causes nearly 3.3 million deaths every year. The National Mental Health Survey of India 2015–2016 have found the prevalence of AUDs to be 9% in adult men and the alcohol-attributable fraction for all cause of deaths was found to be 5.4%.<sup>2</sup> Alcohol abuse has been associated with an increased risk of developing mental and behavioral disorders, a wide range of noncommunicable diseases, and traffic accidents. Apart from the health consequences, it also brings important social and economic losses to affected individuals and countries.<sup>3</sup>

Alcoholism is a heterogeneous disease wherein the influence of genetic vulnerability<sup>4</sup> due to combined effects of multiple genes interacting with other genes<sup>5</sup> is further modified by environmental factors. Mesolimbic dopaminergic system plays an important role in the action of almost all addictive drugs. Its pathway originates in the ventral tegmental area of the midbrain and extends to the nucleus accumbens (NAC), with projections extending to the limbic system and to the orbitofrontal cortex.<sup>6</sup> Alcohol exhibits the primary reinforcing or reward effects by releasing sufficient amount of the pleasure neurotransmitter DA in the NAC. In the search for potential genetic risk factors for alcoholism, various studies have focused on genes associated with ethanol oxidation and neurotransmitter regulation.<sup>7</sup> Functional variants of these genes encoding alcohol-metabolizing enzymes and neurotransmitter signaling molecules in DA and opioid systems contribute interindividual variability toward the development of alcohol dependence (AD).<sup>8</sup> However, in order to identify the determinants of multifactorial diseases such

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**How to cite this article:** Arunachalam U, Chandrasekaran A. Genetic Polymorphism in Alcohol-dependent Genes: A Review. *J Basic Clin Appl Health Sci* 2020;3(1):10–15.

**Source of support:** Nil

**Conflict of interest:** None

as ALD and other alcohol-related disorders, evaluation of functional polymorphism at multiple genes is necessary.<sup>9</sup> Hence, the present review focuses on neurobiology of addiction and various genes associated with AD.

## NEUROBIOLOGY OF ALCOHOL DEPENDENCE

Alcohol dependence poses a serious medical and significant public health problem contributing to morbidity and mortality throughout the world. Despite studies<sup>10,11</sup> suggest the beneficial effects of mild consumption of alcohol, if continues to be on regular basis, it can finally lead to alcohol addiction.<sup>12</sup> The essential features of addiction are characterized by a compulsion to seek and take drug, loss of control in limiting intake, and emergence of a negative emotional state (e.g., dysphoria, anxiety, irritability) when access to the drug is prevented.<sup>13</sup> Much progress in the neurobiology

of addiction can be placed into a heuristic three-stage addiction cycle framework comprising binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation. This framework is supported by multiple neuroadaptations in three corresponding domains: (a) increased incentive salience, (b) decreased brain reward and increased stress, and (c) compromised executive function; and in three major neurocircuits involving basal ganglia, extended amygdala, and prefrontal cortex (Fig. 1). The focus in the neurobiology of addiction has changed with emphasis on the mechanisms of acute reward in the binge/intoxication stage broadened to include neuroadaptations that are consequent to drug exposure. These include mechanisms driving incentive salience, compulsive habits, deficits in reward and recruitment of stress during the withdrawal/negative affect stage, and modulation of executive function systems and mnemonic systems (and being modulated by mnemonic processes) in the preoccupation/anticipation stages of AUDs.<sup>14</sup>

Moreover, discussing about alcohol and adolescent due to rapid development adolescent brain provides increased addiction-related risks. Imaging studies have revealed that the dopaminergic connectivity to the frontal cortex (last region of the brain to develop) is weaker in children than in adults. As the prefrontal cortex matures during adolescence, there is a linear increase in inhibitory control. However, there is also an increased activity in the NAC, which increases reward sensitivity.<sup>15</sup> These differences may contribute to the greater risk-taking behavior, novelty-seeking, and impulsivity that can be observed in adolescence. According to National Longitudinal Alcohol Epidemiologic Survey, 45% of people who began drinking before the age of 14 grew up to ultimately have an AUD, compared with 10% of people who began drinking after the age of 21.<sup>14</sup> The National Epidemiologic Survey on Alcohol and Related Conditions showed that those who are exposed to drinking at an early years of age were more likely to develop AD within a period of 10 years since drinking.<sup>16</sup>

## BIOTRANSFORMATION OF ETHANOL

Alcohol metabolism is a two-step process where the enzyme alcohol dehydrogenase (ADH) oxidizes ethanol to acetaldehyde which is further oxidized to acetate by aldehyde dehydrogenase

(ALDH) (Fig. 2). Accumulation of the toxic intermediate acetaldehyde can cause adverse physiological symptoms including flushing syndrome, tachycardia, and nausea. The rate at which acetaldehyde is produced and converted to the waste product acetate is influenced by genetic variations encoding the isoenzymes of ADH and ALDH. Individuals with isoforms of ADH that oxidize ethanol at a faster rate and/or isoforms of ALDH that oxidize acetaldehyde at a slower rate are protected against AUD due to the unpleasant effects that result from acetaldehyde accumulation.<sup>17</sup>

## ALCOHOL DEHYDROGENASE

The rate-limiting enzyme responsible for the oxidation of ethanol to acetaldehyde is ADH.<sup>18</sup> Apart from ethanol metabolism, ADH is able to metabolize a wide range of substrates such as hydroxysteroids, aliphatic alcohols, lipid peroxidation products, retinoid transformation, etc.<sup>19</sup> Human ADH is unique and exist with polygenic family located on a small region of chromosome 4. There are seven genes that encode the seven known isozymes of ADH whose active forms consist of two subunits.<sup>20</sup> All these seven ADH types have been divided into five classes based on the structural characteristics and the rate at which ethanol is oxidized. Class I comprises three closely related genes *ADH1A*, *ADH1B*, and *ADH1C* that encode  $\alpha$ ,  $\beta$ , and  $\gamma$  subunits which contributes most of

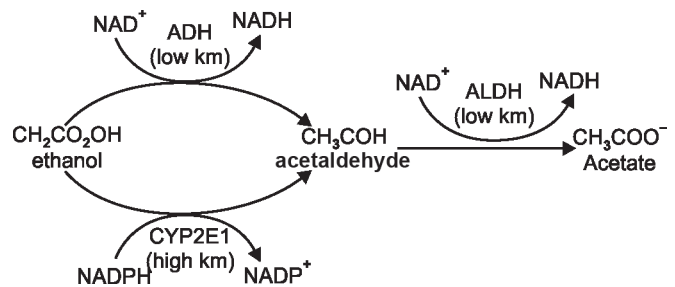


Fig. 2: Pathways of ethanol metabolism in the liver. ALDH, acetaldehyde dehydrogenase; NAD<sup>+</sup>, nicotinamide adenine dinucleotide; NADH, reduced NAD<sup>+</sup>; NADP<sup>+</sup>, nicotinamide adeninedinucleotide phosphate; NADPH, reduced NADP<sup>+</sup>; ADH, alcohol dehydrogenase; *CYP2E1*, cytochromeP-450 isoform 2E1

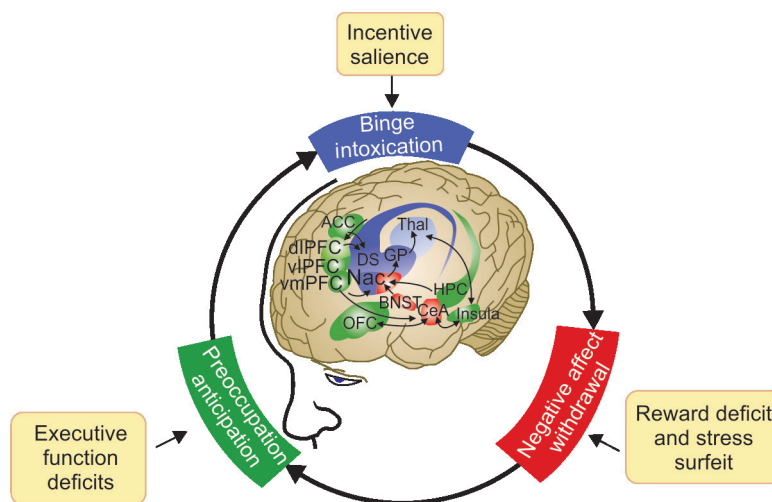


Fig. 1: Conceptual framework for neurobiological bases of the transition to substance-use disorders. PFC, prefrontal cortex; DS, dorsal striatum; GP, globus pallidus; NAC, nucleus accumbens; Hippo, hippocampus; Thal, thalamus; BNST, bed nucleus of the stria terminalis; AMG, amygdala; OFC, orbitofrontal cortex

the ethanol-oxidizing capacity in the liver<sup>21</sup> by the formation of homodimers or heterodimers. Homodimeric proteins are formed by identical subunits coded by the same locus, while heterodimeric proteins are formed by alleles coded from different loci (e.g.,  $\alpha\beta$  and  $\alpha\gamma$ ) or by different alleles at the same locus (e.g.,  $\beta1\beta2$  and  $\gamma1\gamma2$ ).<sup>9</sup> *ADH4* that encodes  $\pi$ -ADH which is predominantly expressed in liver contributes to the metabolism of ethanol at higher concentrations. *ADH5* encodes  $\chi$ -ADH, ubiquitously found in all human tissue, expresses formaldehyde dehydrogenase with very low affinity for ethanol. Not much known about *ADH6*, as the enzyme has not been isolated from any tissue. *ADH7* that encodes  $\sigma$ -ADH involves in ethanol and retinol oxidation, expressed at low level in the liver<sup>22</sup> but present at significant amounts in gastrointestinal tissue. Though different tissues show differentially measurable human *ADH* gene expression, liver contributes a large amount of ADH and expresses the widest number of isozymes, predominantly class I.

Low ADH activity has been demonstrated in the gastric mucosa of females of Caucasian origin and nearly absent in Asians.<sup>23</sup> This feature can increase ethanol blood level which may contribute to the higher susceptibility to ethanol-induced effects in these specified populations. Single-nucleotide polymorphisms (SNPs) located in *ADH1B* and *ADH1C* genes that alter the rate of oxidation of alcohol result in the production of varying quantities of acetaldehyde. *ADH1B* gene demonstrates three alleles that differ in the amino acid sequence of the encoded  $\beta$  subunit. *ADH1B\*1* allele encodes  $\beta1$  subunit that has amino acid arginine (Arg) at positions 48 and 370, *ADH1B\*2* encodes  $\beta2$  subunit that has histidine (His) at position 48 which is frequently seen among Asian population, and *ADH1B\*3* encodes  $\beta3$  subunit that has cysteine (Cys) at position 370.<sup>20</sup> Various studies involving different populations reported that these SNPs shown to play a protective role in the development of AD. Hence, it has been stated that *ADH1B\*2* allele which is found at high frequency among East Asians and *ADH1B\*3* mostly seen in African descent reduce the risk of alcoholism. There are two well-studied variants in *ADH1C*: *ADH1C\*1* encodes  $\gamma1$  subunit that has double variant (Gln272Arg) amino acid arginine (Arg) at position 272 and isoleucine (Ile) at position 350. *ADH1C\*2* encodes  $\gamma2$  subunit that has glutamine (Gln) at position 272 and a valine (Val) at position 350 (Val350Ile). Individuals with *ADH1C\*1* allele have an ethanol-oxidizing capacity of 2.5-times higher when compared to *ADH1C\*2* allele.<sup>24</sup>

## ALDEHYDE DEHYDROGENASE

Acetaldehyde, the toxic metabolite produced by enzymatic ethanol oxidation in the human liver, is further metabolized by ALDH to acetate in a  $\text{NAD}^+$ -dependent reaction. These enzymes have broad substrate specificity for aliphatic and aromatic aldehydes, which are irreversibly oxidized to their corresponding carboxylic acids. The ALDHs are cytosolic enzymes, expressed in a wide range of tissues.<sup>25</sup> A number of isoenzymes of ALDH coded by different gene loci have been detected in humans, which differ in their electrophoretic mobility, kinetic properties, as well as in their cellular and tissue distributions.

Genes coding for ALDH enzymes are divided into nine major families: the major ones are family 1 corresponding to cytosolic ALDHs (*ALDH1*), family 2 to mitochondrial ALDHs (*ALDH2*), family 3 which groups the major constitutive and inducible ALDH forms (*ALDH3*) found in human stomach, saliva, and hepatocarcinoma.<sup>25</sup> Though there are multiple molecular forms of ALDH in human liver, mitochondrial class II ALDH (*ALDH2*) isozyme is predominantly

responsible for the oxidation of acetaldehyde and shown to have high affinity toward it.<sup>26</sup> A coding variant known as the *ALDH2\*2* allele (Glu504Lys, rs671) leads to the production of almost completely inactive *ALDH2* enzyme that does not oxidize acetaldehyde further. Individuals who carry *ALDH2\*1/\*2* (G:A) are heterozygous and shown to have reduced *ALDH2* enzyme activity and individuals who carry *ALDH2\*2/\*2* (A:A) are homozygous and completely lack *ALDH2* enzyme activity.<sup>27</sup> Since the dominant variant *ALDH2\*2* allele is relatively common in Chinese, Japanese, and Korean population, alcohol flush reaction or aldehyde syndrome have been reported even on minimal consumption of alcohol.<sup>29</sup> Hence, *ALDH2\*2* allele provides protection against the development of AD which is not seen in people of European or African descent<sup>28</sup> as they lack the allele. Considering the effects of *ALDH2\*2* variant on the risk of AD, mitochondrial *ALDH2* enzyme is responsible for maintaining acetaldehyde levels extremely low.<sup>20</sup> When combined, both the ADH and *ALDH2* variants are highly protective against the risk of developing AUD.<sup>30</sup>

## CYTOCHROME P450 2E1

Microsomal ethanol oxidation system (MEOS) is involved in ethanol oxidation by the cytochrome P450 (CYP) enzymes, which include *CYP2E1*, *CYP1A2*, and *CYP3A4* isoforms.<sup>31</sup> Cytochrome *P450 2E1* (*CYP2E1*) is normally a minor pathway accounting for approximately 10% of ethanol oxidation, but may be inducible at high ethanol levels up to 10-folds.<sup>32</sup> The induction or higher basal activity of *CYP2E1* has been suggested to result in faster ethanol inactivation during long-term alcohol consumption, which may further increase one's motivation to consume more alcohol and thereby contribute to the development of AD.<sup>33</sup>

The *CYP2E1* gene has been localized to chromosome 10 and consists of 9 exons and 8 introns, encoding a 493-amino acid protein. Ten polymorphic loci on human *CYP2E1* gene have been reported so far, most of them are in the promoter and intron regions. In addition, a tandem repeat was identified in *CYP2E1* regulatory region.<sup>34</sup> The *RsaI* restriction fragment length polymorphism has been found within the *CYP2E1* gene (c.-1053C>T, rs2031920). Various linkage and association analyzes show that sequence changes within or near the *CYP2E1* gene affect the level of response to alcohol, providing a predictor of alcoholism risk.<sup>9</sup> The rare mutant allele (c2 allele) lacking the *RsaI* restriction site has been shown to be associated with higher enzymatic activity and protein levels than the wild-type *c1* allele. Moreover, the frequency of *RsaI* c2 allele varies in different populations: the highest frequency has been observed in the Taiwanese (28%) and Japanese populations (19–27%), while the frequency is much lower (1–5%) in Africans.<sup>25</sup> The enhanced transcriptional activity of *CYP2E1* c2/c2 might play a role in the development of severe ALD.<sup>35</sup> In a study involving Mexican-American men, strong association was found between *CYP2E1* *RsaI/DraI* genotype and alcoholism. *CYP2E1* *RsaI* c2 and *DraI* C allele frequencies are found to be much higher in alcoholics than in nonalcoholics (26.4% vs 9.6% for c2 and 27.8% vs 13.5% for C allele).<sup>36</sup> Hence, *CYP2E1* c2/C alleles may contribute to the development of alcoholism in Mexican-American men. Soya et al. were the first to report genotype and allele frequencies of genes *CYP2E1\*1B*, *CYP2E1\*6* and *CYP2E1\*5B* among Tamilian population.<sup>37</sup> They noticed similar pattern of distribution in genotype when compared with north Indian population.



## OPIOID RECEPTOR

There are three main classes of opioid receptors:  $\mu$  (MOR),  $\delta$  (DOR), and  $\kappa$  (KOR), belonging to G-protein-coupled receptor family which are the site of action of major opioid peptides like  $\beta$ -endorphin.<sup>4</sup> The human MOR gene, *OPRM1* located on chromosome 6(q24-q25), consists of at least nine exons and spans over 200 kb.<sup>38</sup> The most studied and most abundant variant, MOR-1, spans about 80 kb and contains four exons. One functional SNP, rs1799971, occurs at position 118 within exon 1 of the *OPRM1* gene. Here, an adenine-to-guanosine substitution (A118G) leads to an exchange of an asparagine for an aspartic acid at a putative glycosylation site (N40D) in the extracellular loop of the receptor.<sup>39</sup> The A118G SNP is common in individuals of European (15–30%) and Asian ancestry (40–50%), while rare in individuals with African or Hispanic heritage (1–3%).<sup>40</sup>

Human and animal studies implicate the opioid system, mediating initial response as well as rewarding effects of alcohol predominantly through the  $\mu$ -opioid receptor. It has been observed that baseline plasma  $\beta$ -endorphin levels were lower in high-risk individuals for alcoholism, but on exposure to ethanol shown to be a more pronounced release.<sup>41</sup>

Single-nucleotide polymorphism, rs1799971, located in the  $\mu$ -opioid receptor gene (*OPRM1*) is shown to increase the binding affinity of  $\beta$ -endorphins to the receptor. Bart et al. reported an association of the 118G allele in exon1 of the MOR (*OPRM1*) gene to AD, independent of type I and type II characteristics.<sup>42</sup> According to this study, attributable risk for AD in subjects from central Sweden with 118G allele was 11.1%. However, Van Der Zwaluw et al. reviewed about 12 case control studies and showed that clinical studies do not unequivocally support an association between polymorphisms in *OPRM1* and AD. Xiangyi Kong also found that rs1799971 (A > G) is not strongly associated with alcohol dependence. According to Annika Thorsell, genetic variants are important when considering treatment options for different individuals and play a crucial role in determining which patients will be most likely to respond to naltrexone treatment. Benefits of such pharmacogenetic approach decrease the risk of exposing individuals to ineffective medication and thereby increase treatment response and health. Oslin confirmed that individuals with one or two copies of the Asp40 allele treated with naltrexone had significantly lower rates of relapse and taken longer time to return to heavy drinking.<sup>43</sup> Chamorro et al. also support that the genetic marker G allele of A118G polymorphism of *OPRM1* can be used to identify a subgroup of individuals more likely to respond to this treatment.<sup>44</sup>

Deb et al. demonstrated the involvement of A118G polymorphism of exon1 of human *OPRM1* gene with heroin and alcohol addiction, among East Indian population. In this study, the allelic frequencies of A118G for A and G in three different groups were 58% and 42% in opioid-dependent subjects, 60% and 40% in alcoholic subjects, and 72% and 28% in the control population, respectively, and also stated A118G as a possible risk factor for addiction toward narcotics and alcohol.<sup>45</sup>

## DOPAMINE RECEPTOR

Dopamine (DA) is considered to be an important neurotransmitter since it involves in reward and motivation mechanisms of the brain. Though the primary site of action of alcohol is different, ultimately there is increase in the level of DA leading to the development

and relapse of AD.<sup>46</sup> Variant of DA D2 receptor DRD2 gene located on chromosome 11 found likely to be associated with higher consumption of alcohol. Three most commonly studied polymorphisms at DRD2 locus in association with AD are TaqIA, TaqIB, and -141C Ins/Del.<sup>47</sup> Promoter polymorphism (-141C ins/Del, rs1799732) of the DRD2 gene involving the insertion (Ins)/deletion (Del) of a cytosine is related to receptor density, whereas SNP TaqI B is closer to the regulatory and structural coding regions (5'-region) of the gene and plays an important role in transcription regulation. However, the Taq I A site of the ANKK1 gene is widely used in the studies related to substance abuse and addiction. The restriction of Taq I results in polymorphic fragments identifying the alleles namely A1 and A2 which have a difference in the number of their receptor sites. Earlier studies have shown that the people having A1 allele are more prone to alcohol dependency as the A1 allele decreases the expressivity of D2 DA receptors.<sup>48</sup> Family-based and case-control study confirmed that dopaminergic system polymorphic variants DRD2 -141C Ins/Del in the promoter region and DRD2 TaqI A are partially responsible for the development of AD.<sup>49</sup> Prasad et al. reported the existence of significant association between genetic polymorphisms in DRD1 and DRD4 and alcoholism. They also reported the frequency of allele Ins and Del as 0.77 and 0.23, respectively, in another study and stated that two polymorphisms -141C Ins/Del and TaqI A in DRD2 gene may influence the development of AD among Indian alcoholic subjects. Similar results were observed when Sinha et al. conducted a study with alcohol-dependent males of central India origin. Suraj Singh et al. also found that the individuals possessing both the A1 and the-141C Del alleles, if consuming alcohol at an early age, may be at higher risk of AD.

## FUTURE PROSPECTS

Alcohol-use disorders impose huge health and economic burdens on individuals, families, communities, and society. Though preventive and treatment measures are available, efforts are not effective in all individuals and the outcome is not promising. Alcohol-use disorder prevention could be enhanced with a growing knowledge of the disorder's neurobiology and genetics. Increasingly sophisticated genetic tools (haplotype and SNP maps, mapping arrays, expression arrays) are being applied to complex diseases which will improve both the understanding of at-risk individuals' biology and the development of new medications. The success for naltrexone in the treatment of AD depends in part on a polymorphism in the  $\mu$ -opioid receptor, and this gives the possibility of genotype-based selection of pharmacotherapy for alcoholism.<sup>50</sup> Another important application of genetic medicine is selection of biomarkers for alcohol and drug dependence based on changes in gene expression or protein levels in blood samples. Those sensitive and selective biomarkers can only be defined after measuring many different transcripts or proteins with array technologies. The immediate future may bring the realization to define the genetics of dependence, and better understanding about how genes interact with environmental variables to influence drug responses and related behaviors is needed.

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