ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE GENE POLYMORPHISM IN TURKISH ALCOHOLIC PEOPLE AND CONTROL GROUP

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ESİN SALMAN

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İZMİR

We approve the thesis of **Esin SALMAN**

Date of Signature

..... **20 July 2007**
Assoc. Prof. Dr. Oğuz BAYRAKTAR
Supervisor
Department of Chemical Enginering
İzmir Insitute of Technology

..... **20 July 2007**
Prof. Dr. Ulus Salih AKARCA
Co-Supervisor
Department of Gastroenterology
Ege University Medical Faculty

..... **20 July 2007**
Assoc. Prof. Dr. Anne FRARY
Department of Biology
İzmir Institute of Technology

..... **20 July 2007**
Assist. Prof. Dr. Ekrem ÖZDEMİR
Department of Chemical Enginering
İzmir Institute of Technology

..... **20 July 2007**
Assist. Prof. Dr. Ayşegül BATIGÜN
Department of Chemical Enginering
İzmir Institute of Technology

..... **20 July 2007**
Prof. Dr. Semra ÜLKÜ
Head of Department
İzmir Institute of Technology

……
Prof. Dr. M. Barış ÖZERDEM
Head of Graduate School

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ABSTRACT

ALCOHOL DEHYDROGENASE AND ALDEHYDE DEHYDROGENASE GENE POLYMORPHISM IN TURKISH ALCOHOLIC PEOPLE AND CONTROL GROUP

In this study, alcohol dehydrogenase and aldehyde dehydrogenase gene, encoding alcohol metabolizing enzymes, polymorphisms were determined in alcoholic and non-alcoholic Turkish people by using effective, commonly used single nucleotide polymorphism determination methods. The main objective in the study was to investigate the relationship of ADH2, ADH3 and ALDH2 gene polymorphism with the tendency of Turkish people to develop alcohol tolerance. The other significant objective was to compare polymorphism types of ADH2, ADH3 and ALDH2 seen in Turkish people and other ethnic groups or races in the world. In this present study, ADH3 genotypes of 141 alcoholic subjects, and also ADH2 and ALDH2 genotypes of 156 alcoholic subjects were assigned. The control group consisted of 80 healthy non-drinkers. Three different SNP genotyping methods were used in this study. ADH3 genotyping was performed using polymerase chain reaction-restriction fragment length polymorphism method (RFLP-PCR). ADH2 genotyping was performed by allele specific primer extension method and ALDH2 genotyping was performed by multiplex PCR by using two allele specific primer pairs. The ADH2.1 genotype was the most common type of all ADH2 genotypes in both alcoholic and non alcoholic groups. However, there was no significant difference between alcoholic and non alcoholic groups for ADH2 genotyping. ADH3 genotyping of both groups suggested that the ADH3.2 genotype frequency was higher than ADH3.1. ADH3.2 was found to be more prevalent in alcoholics compared to control group, suggesting that alcoholics were more tolerant to alcohol. In all of the alcoholic and non-alcoholic subjects examined, the frequency of ALDH2.1 was found to be 100%. Finally, it can be inferred from that obtained results, ADH2 genetic variations seem not to be related to alcoholism. On the other hand, ADH3 and ALDH2 genetic variations can make Turkish people susceptible to alcohol dependency. If all the results are taken into consideration, it is inferred that Turkish people have the inherited variations of ADH and ALDH genes which do not protect them to have alcohol sensitivity and dependency. Obtained results in the study are consistent with the white race in the world including European people but not consistent with Oriental people as expected.

ÖZET

TÜRK ALKOLİKLERDE VE KONTROL GRUBUNDA ALKOL DEHİDROGENAZ VE ALDEHİT DEHİDROGENAZ GEN POLİMORFİZMİ

Bu tez çalışmasında alkolik ve alkolik olmayan Türklerde alkol metabolizmasında önemli rol oynayan enzimleri kodlayan alkol dehidrogenaz ve aldehit dehidrogenaz genlerinin polimorfizmleri günümüzde en yaygın kullanılan Single Nükleotid Polimorfizm (SNP) belirleme metodları kullanılarak belirlenmiştir. Çalışmadaki temel amaç, ADH2, ADH3 ve ALDH2 gen polimorfizmleri ile Türk ırkının alkole karşı tolerans geliştirme eğilimi arasındaki ilişkiyi saptamaktır. Diğer önemli amaç ise, Türk ırkındaki ADH2, ADH3 ve ALDH2 genlerine ait polimorfizm tiplerini dünyadaki diğer ırklarla ve etnik gruplarla karşılaştırmaktır. Çalışmada 141 alkolik hasta için ADH3 genotiplemesi ve 156 alkolik hasta için de ADH2 ve ALDH2 genotiplemeleri yapılmıştır. Kontrol grubu ise 80 sağlıklı alkol kullanmayan bireyden oluşmuştur. Bu çalışmada üç farklı SNP genotipleme metodu kullanılmıştır. ADH3 genotiplemesi için RFLP-PCR, ADH2 genotiplemesi için allel spesifik primer extension metodu, ALDH2 genotiplemesi için ise Multiplex PCR metodu kullanılmıştır. Alkolik ve alkolik olmayan gruplarda tüm ADH2 genotipleri içinde en yaygın olarak ADH2.1 genotipi bulunmuştur. Bununla birlikte ADH2 genotipleri açısından genel olarak kontrol grubuyla alkolik grup arasında anlamlı bir fark bulunmamıştır. İki grup içinde yapılan ADH3 genotiplemesinde ADH3.2 genotipi oranının ADH3.1 genotipi oranından yüksek olduğu bulunmuştur. Alkolik grupta ADH3.2 genotipinin kontrol grubundan yüksek olduğu bulunmuştur. İncelenen alkolik grup ve kontrol gruplarının her ikisinde de ALDH2.1 genotipi %100 oranında saptanmıştır. Sonuç olarak, ADH2 genindeki varyasyonların Türklerde alkolizme katkısının olmadığı, buna karşılık ADH3 ve ALDH2 genlerindeki varyasyonların Türk insanını alkol bağımlılığı geliştirmeye karşı eğilimli yapabileceği söylenebilir. Tüm sonuçlar gözüne alındığında ise Türk ırkının ADH ve ALDH genlerinin alkole karşı hassasiyeti ve alkol bağımlılığını arttırma eğiliminde olan genetik varyasyonlarına sahip olduğu çıkarımı yapılabilir. Elde edilen sonuçlar Asya toplumununkinden önemli ölçüde farklı ve beklendiği üzere Avrupa toplumu dahil dünya genelindeki beyaz ırkla uyumlu bulunmuştur.

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CHAPTER 1

INTRODUCTION

Ethanol is a kind of drug affecting the mind, mood and other mental processes. Consumption of ethanol is very common throughout the world. Uncontrolled or excessive intake of alcoholic beverages is defined as alcoholism. Alcoholism is a public concern for many countries, because it influences physical or mental health, social and familial relationships, and occupational responsibilities (Sun et al. 2002, Chinnaswamy et al. 2005).

Individuals give different responses when exposed to comparable amount of alcohol. Alcoholism must be accepted as a chronic disorder with a complex origin and outcome (Gemma et al. 2006). To date, the relationship between alcohol consumption and fatty liver and also alcoholic hepatitis and cirrhosis have been investigated efficiently. The reason behind the liver diseases based on excessive and prolonged alcohol consumption is that liver is the main organ responsible for alcohol metabolism (Tekin et al. 2005).

Alcoholism is an important cause of chronic liver diseases, but only 10% to 20% of alcoholics develop cirrhosis (Lee et al. 2001). While a group of drinkers do not develop cirrhosis or other chronic liver diseases, other groups who possibly consume less alcohol can have considerable liver damage. Individual based genetic variations in the genes encoding the enzymes playing active role in ethanol metabolism are considered to be responsible for this difference (Tekin et al. 2005).

Although alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH) and microsomal P4502E1 (CYP2E1) are the hepatic enzymatic systems involved in ethanol metabolism, ethanol is mostly metabolized in the liver by the contributions of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH).

Alcohol is oxidized to acetaldehyde by the alcohol dehydrogenase enzymes (ADHs), and acetaldehyde is further oxidized to acetate by the aldehyde-dehydrogenase enzymes (ALDHs). Both enzymes exhibit genetic polymorphisms (Borras et al. 2000).

Acetaldehyde, a highly toxic metabolite of ethanol, has a very significant effect on the pathogenesis of alcoholic liver disease and alcohol dependency (Lee et al. 2001). Studies prove that accumulation of acetaldehyde in blood induces unpleasant effects

such as flushing, headache, tachycardia and hypotension. Indeed, because of being limiting factors for further drinking, these symptoms can prevent alcohol dependence considerably (Tekin et al. 2005).

This case suggests that genetically determined variations in an individual's ability to metabolize alcohol might influence the prevalence of alcoholic liver diseases (Lee et al. 2001).

Overall risk for alcohol- related adverse events, alcohol dependence and alcohol related liver diseases are strongly related to genetic tendency and environmental factors such as nutrition and lifestyle (Ehlers et al. 2001). For this reason, alcoholism must be examined as a multifactorial disease depending on genetic-environmental interaction and genetic variations (Chinnaswamy et al. 2005).

The studies about alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genes polymorphism have been carried out in different races including Turkish people living abroad since many years. However, up to now, the study done in Turkey is limited except for one where only ADH3 polymorphism was examined.

In this study, the objective was to investigate the correlation among ADH2, ADH3 and ALDH2, encoding alcohol metabolizing genes, polymorphisms and the tendency of Turkish people to develop alcohol tolerance. The other significant goal was to compare variants of ADH2, ADH3 and ALDH2 genes in Turkish people and other ethnic groups or races in the world.

CHAPTER 2

ETHANOL METABOLISM AND ALCOHOLISM

2.1. Physical Properties of Ethanol

Ethanol, CH_3CH_2 OH, is uncoloured, water-miscible organic solvent, with a characteristic smell. Ethanol molecules include a hydroxyl group (– OH) bonded to a carbon atom. Melting temperature for ethanol is –114.1°C, boiling temperature for ethanol is 78.5°C. Also, ethanol has a density of 0.789 g/mL at 20°C (WEB_7, 2007).

Ethanol is a psychoactive drug, but, besides its pharmacologic action, it can be an energy source for body since it has energy, 7.1 kcal/g (Lieber et al. 2000, WEB 2, 2007).

2.2. Ethanol Metabolism

Ethanol has been consumed as a beverage in the world for centuries. Excessive consumption of ethanol can be a reason for some serious health problems. To understand the biochemical basis of these health problems, ethanol metabolism pathway should be examined. The vast majority of ethanol metabolism occurs in the liver (Chinnaswamy et al. 2005).

2.2.1. ADH and ALDH

The metabolism of ethanol is divided into two main pathways. The first pathway consists of two steps. The first step, catalyzed by the alcohol dehydrogenase (ADH), takes place in the cytoplasm:

$$\underset{\text{Ethanol}}{CH_3CH_2OH} + NAD^+ \xrightarrow{\text{Alcohol dehydrogenase}} \underset{\text{Acetaldehyde}}{CH_3CHO} + NADH + H^+$$

Approximately, 90% of ethanol conversion to acetaldehyde takes place in cytoplasm.

The second step, catalyzed by aldehyde dehydrogenase, takes place in mitochondria:

$$\underset{\text{Acetaldehyde}}{CH_3CHO} + NAD^+ + H_2O \xrightarrow{\text{Aldehyde dehydrogenase}} \underset{\text{Acetate}}{CH_3COO^-} + NADH + H^+$$

2.2.2. Microsomal Ethanol-Oxidizing System (MEOS)

The second pathway for ethanol metabolism is called the ethanol inducible microsomal ethanol-oxidizing system (MEOS). This cytochrome P450-dependent pathway generates acetaldehyde and immediately after acetate while oxidizing biosynthetic reducing power, NADPH, to $NADP^+$. Only 10% of ethanol conversion to acetaldehyde takes place in cytoplasm.

Figure 2.1 shows the role of cytochrome P-450 isoform 2E1 in detail together with metabolic functions of ADH, ALDH and Catalase.

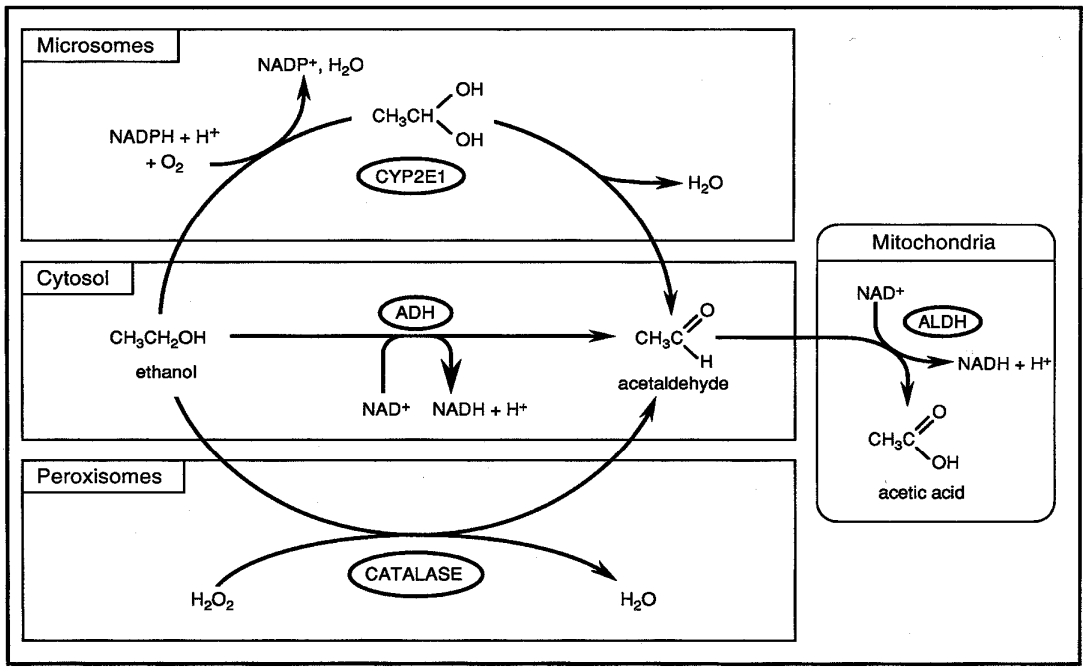

Figure 2.1. Pathways of Ethanol Metabolism in the Liver.

2.2.3. Catalase

Catalase is also involved in the process of alcohol oxidation (Cichoz et al. 2007). But, the reaction catalyzed by Catalase can be ignored beside the other effective pathways (Tekin et al. 2005). On the other hand, in excessive ethanol consumption, Catalase can play an active role in the conversion of ethanol to acetaldehyde.

2.3. Ethanol Metabolites and Liver Damage

Since the major parts of ethanol metabolization take place in it, the liver is an organ that is especially susceptible to toxic effects from metabolites of ethanol (Cichoz et al. 2007). Ethanol consumption is responsible for an accumulation of NADH. NADH has an inhibition effect on fatty acid oxidation pathway. So, excessive NADH amounts created by ethanol metabolization trigger fatty acid synthesis (Figure 2.2.). As a result, triacylglycerols accumulate in the liver. Finally, this case gives rise to a condition is known as fatty liver.

Another destructive ethanol metabolite for liver is acetaldehyde. If processing of the acetate in the liver becomes inefficient, acetaldehyde accumulation occurs in the blood. Acetaldehyde has the ability to impair protein functions. Because, it is a very reactive compound forming covalent bonds with many important functional groups in proteins. If ethanol is continually consumed at high levels, the acetaldehyde can significantly damage the liver by causing cell death.

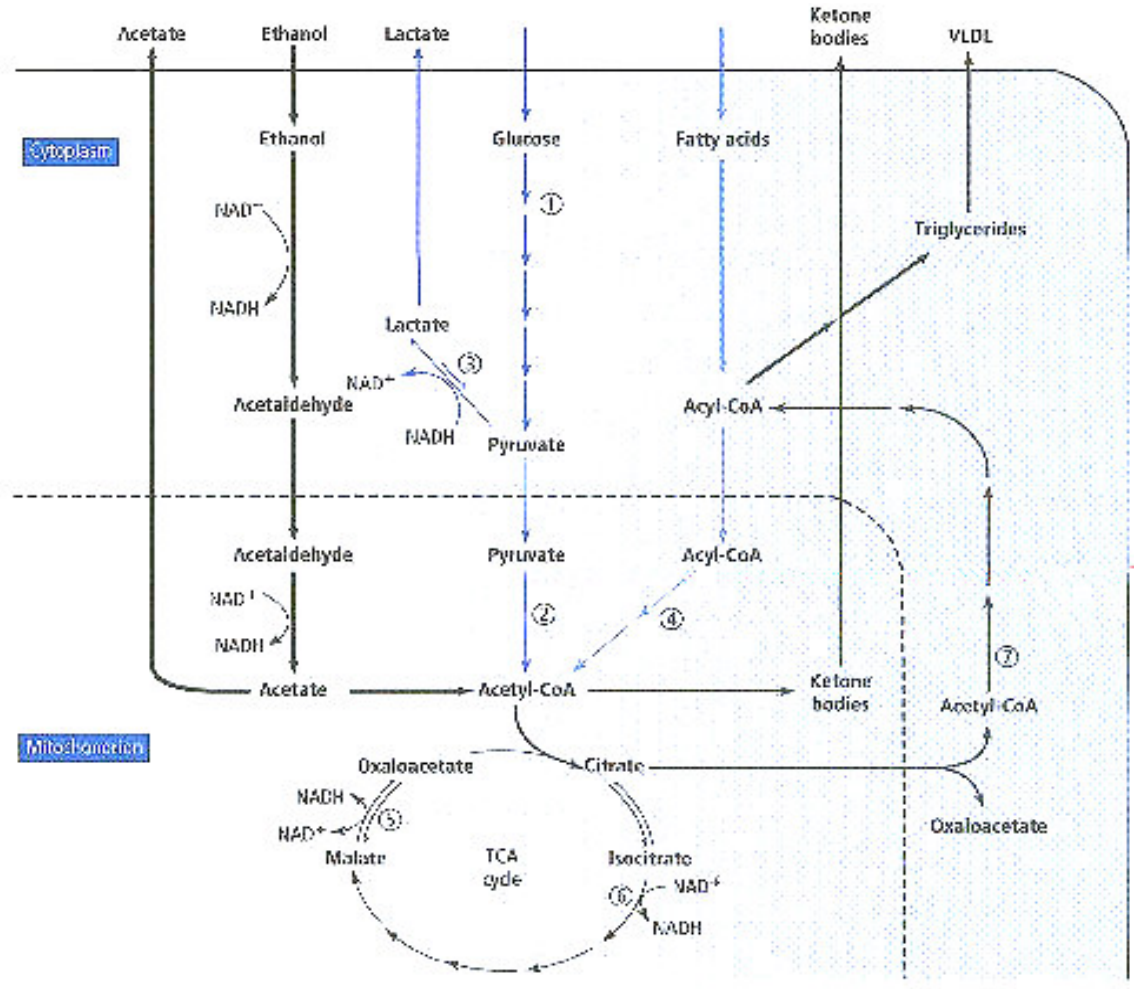

Figure 2.2. Metabolic Pathways within the Liver Cell.
(Source: WEB_2, 2007)

Development of liver damage related to excessive ethanol consumption divided into three stages. The first stage is the development of fatty liver. In the second stage, alcoholic hepatitis takes place. This stage can itself be fatal by causing cell death and inflammation. In the third stage, cirrhosis occurs. Cirrhosis is characterized with fibrous structure and scar tissue is produced around the dead cells in liver. Cirrhosis impairs many of the liver's biochemical functions. It is seen in about 25% of alcoholics, and about 75% of all cases of liver cirrhosis are the result of alcoholism (Berg et al. 2002). In Figure 2.3., the stages of alcoholic liver diseases and their effects are shown.

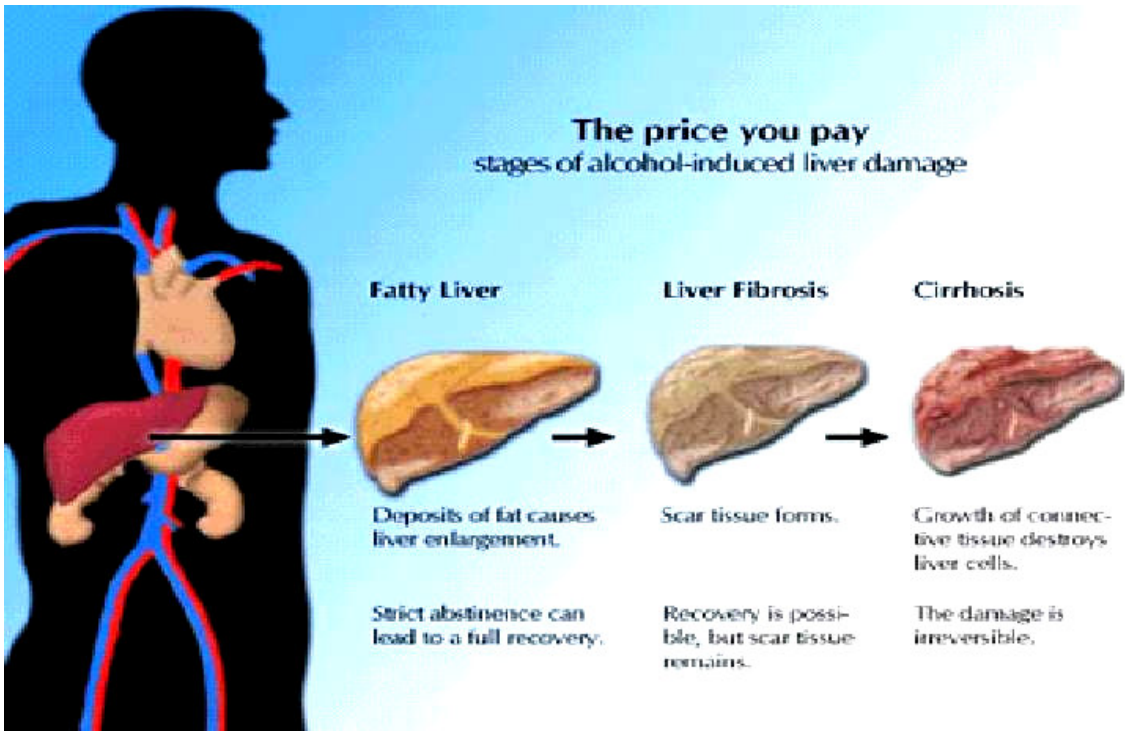

Figure 2.3. The Stages of Alcoholic Liver Diseases.

2.4. ADH and ALDH Genes

Ethanol metabolism enzymes have been the focus of a great deal of study. Starch gel electrophoresis of human liver extracts reveals a complex pattern of alcohol (ADH) and aldehyde dehyrdrogenase (ALDH) isoenzymes (Crabb et al. 1990).

2.4.1. ADH

Alcohol dehydrogenase (EC.1.1.1.1) β subunit is an enzyme that converts ethanol to acetaldehyde, whose gene, ADH2 located in chromosome 4q22 (Chinnaswamy et al. 2005).

2.4.1.1. ADH Isoforms

As many as 7 ADH genes have been identified in humans (Figure 2.4.). ADH isoforms that are important for ethanol metabolism are the Class I, Class II, and Class

IV isozymes (Li et al. 2001). Table 2.1 shows the polymorphic ADH gene types in detail.

Figure 2.4. ADH Gene Cluster.
(Source: Luo et al. 2006)

Enzymatically active ADH is composed of two protein subunits. All Class I isozymes are found in liver, and consist of homo- and heterodimeric forms of the three α, β and γ subunits. (ie., αα, αβ, ββ, βγ, etc.). The corresponding genes are ADH1, ADH2, and ADH3. The Class II ADH is a single homodimeric enzyme ππ found in liver, and the Class IV enzyme is a single homodimeric enzyme σσ, found primarily in stomach (Li et al. 2001).

There are functionally different forms of the β and γ subunits encoded by variant alleles of the ADH2 gene and the ADH3 gene. Thus the ADH 2.1, ADH 2.2 and ADH 2.3 alleles give rise to β_1, β_2 and β_3 subunits, respectively, and the ADH3.1 and ADH3.2 alleles give rise to the γ_1 and γ_2 subunits, respectively. All these subunits have different catalytic activity or ability for ethanol oxidation. The polymorphic genes ADH2 and ADH3 encode different forms of β and γ subunits of different features each (Li et. al. 2001).

ADH2 has a functional polymorphism Arg47His (Tamakoshi et al. 2001). In the ADH2.2 (atypical) allele, CAC codes for Histidine at residue 47; in the corresponding codon of the ADH2.1 (typical) allele, CGC codes for Arginine. The enzyme activity is higher in the 47His allele (ADH2.2) than in the 47Arg allele (ADH2.1) (Chinnaswamy et al. 2005). ADH2 gene is also polymorphic at residue of 369, a change from Cytosine to Thymine causes to form Cysteine in place of Arginine (ADH2.3). However, only ADH2 (Arg47His) has been routinely tested in association studies with alcoholism (Osier et al. 2002).

ADH3 gene is polymorphic at residue of 349. A single nucleotide change from Guanine to Adenine at residue 349 causes to form Val (Valine) in place of Ile (Isoleusine) (Osier et al. 2002).

Table 2.1. Polymorphism of Alcohol Dehydrogenase (ADH) gene.
(Source: Gemma et al. 2006)

Class (Protein)	Gene	Subunit	Nucleotide change	Effect	Chromosomal location
Class I ADH	ADH1*1	α		wild -type	4q22
ADH1					
ADH2	ADH2*1	β1		wild -type	4q22
	ADH2*2	β2	47 G>A	His^{47} increased V_{max}	
	ADH2*3	β3	369C>T	Cys^{369} increased V_{max}	
ADH3	ADH3*1	γ1		wild -type	4q22
	ADH3*2	γ2	271C>T;349G>A	Gln^{271};Val^{349}	
Class II ADH	ADH4	Π	192T>A;159G>A; 75A>C	altered expression	4q21-25
ADH4					
Class III ADH	ADH5	X		wild -type	4q21-25
ADH5					
Class IV ADH	ADH7	σ		wild -type	4q23-24
ADH7					
Class V ADH	ADH6	?*		?*	4q21-25
ADH6					

* Subunit composition not known.

2.4.2. ALDH

Aldehyde dehydrogenase (EC.1.2.1.3) catalyzes acetaldehyde the NAD^+-dependent oxidation of acetaldehyde and other aliphatic aldehydes into acetate, whose gene, ALDH2 is in 12q24.2 chromosome (Crabb et al. 1989, Li et. al. 2001).

2.4.2.1. ALDH Isoforms

As many as 12 ALDH genes have been identified in humans. ALDH isozymes important for acetaldehyde metabolism are the mitochondrial ALDH2 and one of the cytosolic aldehyde dehydrogenases, ALDH1 (Li et al. 2001). Mitochondrial aldehyde

dehydrogenase (ALDH2) is a tetrameric protein which has multiple molecular forms in human liver (Crabb et al. 1989).

ALDH2 gene has a G-to-A polymorphism in the residue of 1510. This single nucleotide change results in Glu487Lys.This polymorphism is very important for function of ALDH2. The 487Glu (ALDH2.1) allele has full enzyme activity, whereas the 487Lys allele (ALDH2.2) has no activity. It means that individuals with 487Lys/Lys genotype are unable usually to drink a glass of beer, because of the impairment of acetaldehyde detoxification (Tamakoshi et al. 2003, Crabb et al. 1989).

ALDH2 gene is also polymorphic at residues 1486 and 1464. While a change in residue of 1486 from Guanine to Adenine results in to form 479Lysine allele in place of Glutamic acid allele (ALDH2.3), a change in residue of 1464 from G to A results in no aminoacid change (silent). In Table 2.2, polymorphic ALDH gene types are examined in detail.

Table 2.2. Polymorphism of Aldehyde Dehydrogenase (ALDH) Gene.
(Source: Gemma et al. 2006)

Locus	**Gene**	**Nucleotide change**	**Effect**	**Chromosomal location**
ALDH1B1	ALDH1B1*1		wild-type	9p13
	ALDH1B1*2	257C>T	Ala^{69}	
	ALDH1B1*3	320T>G	Leu^{90}	
	ALDH1B1*4	183C>T	silent	
ALDH2	ALDH2*1		wild-type	12q24.2
	ALDH2*2	1510G>A	Lys^{487}	
	ALDH2*3	1486G>A	Lys^{479}	
	ALDH2*4	1464G>A	silent	
ALDH3A1	ALDH3A1*1		wild-type	17p11.2
	ALDH3A1*2	985C>G	Ala^{329}	
ALDH3A2	ALDH3A2*1		wild-type	17p11.2
	ALDH3A2*2	521T del	frameshift	
	ALDH3A2*3	808Gdel	frameshift	
	ALDH3A2*4	941 del 3bp, 941 ins 21 bp	Gly^{314},Ala^{315}, 6aa ins	
	ALDH3A2*5	641G>A	Tyr^{214}	
	ALDH3A2*6	1297GA del	frameshift	
	ALDH3A2*7	1311 ins 5 bp	frameshift	
	ALDH3A2*8	1297GA del	frameshift	
ALDH4A1	ALDH4A1*1		wild-type	1
	ALDH4A1*2	21G del	frameshift	
	ALDH4A1*3	1055C>T	Leu^{352}	
	ALDH4A1*4	47C>T	Leu^{16}	
	ALDH4A1*5	1563T ins	frameshift	
ALDH9A1	ALDH9A1*1		wild-type	1q22-q23
	ALDH9A1*2	344G>C	Ser^{115}	
	ALDH9A1*3	327C>T	silent	

2.5. Alcoholism and Genetic Tendencies

2.5.1. Genetic Aspects of Alcoholism

Many biomedical and psychosocial studies prove that some individuals have more sensitivity to alcohol. It means that, experienced adverse effects following alcohol use can vary from individual to individual (Gemma et al. 2006). Also, while some individuals have more tendency to develop alcoholism, some people have the susceptibility to alcohol dependency. Alcoholism is a chronic disease depending on genetic-environmental interaction and genetic variations (Chinnaswamy et al. 2005). Thus, overall risk for alcohol- related adverse events, alcohol dependence and alcohol related liver diseases are strongly related to both genetic tendency and environmental factors (Ehlers et al. 2001).

2.5.2. The Effects of Alcohol Metabolizing Gene Polymorphism on Alcoholism

Alcohol dehydrogenase (ADH) and mitochondrial aldehyde dehydrogenase (ALDH2) encoding genes are responsible for ethanol metabolism. Alcohol dehydrogenase and mitochondrial aldehyde dehydrogenase have multiple isozymes that have different kinetic characteristics (Ehlers et al. 2001).

In humans, genetic polymorphisms of alcohol dehydrogenase and aldehyde dehydrogenase encoding genes are associated with alcohol drinking habits and the density of alcohol abuse (Quertemont et al. 2001).

Current molecular genetic investigations have suggested that polymorphisms at these genes encoding alcohol dehydrogenase (ADH) and mitochondrial aldehyde dehydrogenase (ALDH2) determine ethanol toxicity in the body. Also, diversity of susceptibility in alcohol-induced toxic effects, alcohol abuse or alcohol based diseases are strongly related to ethanol-induced toxic effects (Gemma et al. 2006). Some genetic studies have suggested that accumulation of acetaldehyde, first metabolite for ethanol metabolism, in blood is responsible for some disagreeable adverse effects following alcohol intake such as vomiting, flushing, hypotension. Acetaldehyde metabolization

impairment results from insufficient functions of ethanol metabolism enzymes and this case can make a contribution to alcohol abuse and alcoholism (Quertemont et al. 2001).

The risk of development of alcohol related disorders is strongly associated with positive family history of alcoholism. First-degree relatives of alcoholics are under the risk seven times more likely than the general population (Ehlers et al. 2001).

Family studies on twins also estimated that individual risk for alcoholism can be equally addressed to environmental and genetic factors which show a high degree of interaction (Gemma et al. 2006). Since the frequency of ADH and ALDH coding genes differs across ethnic groups, alcohol dependency or alcohol related health problems have interethnic differences (Ehlers et al. 2001, Gemma et al. 2006).

2.6. Alcohol Dehydrogenase and Aldehyde Dehydrogenase Gene Polymorphism

Current studies on monozygotic and dizygotic twins, family based groups estimated that alcoholism is received by inheritance in a range of 50–60%. In other words, susceptibility to alcoholism is strongly related to inherited variation in genes, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) which have the major parts of ethanol metabolism in the human body (Roberts et al. 2004, Vidal et al. 2004). The rates of pathways that are responsible for conversion of alcohol to acetaldehyde and acetaldehyde to acetate are mostly influenced by functional polymorphisms on ADH2, ADH3, and ALDH2 (Roberts et al. 2004).

In humans, three polymorphisms related to alcohol conversion have been identified for ADH2 (*1,*2,*3), two for ADH3 (*1,*2), and two for ALDH2 (*1,*2) (Wall et al. 2003).

2.6.1. ADH2 and ADH3 Genes Polymorphism and Alcoholism

Studies about ADH gene polymorphisms concluded that the alleles of ADH2.2, ADH3.1 are most likely to play a protective role against excessive alcohol consumption. Especially, strongly association between reduced alcohol consumption or reduced risk of alcohol dependence and the ADH2.2 variant allele has recently been found.

ADH2.2 and ADH3.1 are the alleles associated with higher ADH activity and they increase the offensive effects of alcohol. However, ADH2.1 and ADH3.2 are alleles associated with slower ADH activity and they increase alcohol tolerance and promote alcoholism (Roberts et al. 2004). As seen from the Figure 2.5., polymorphisms that promote higher levels of acetaldehyde with lower intakes of alcohol are associated with lower risks for alcoholism.

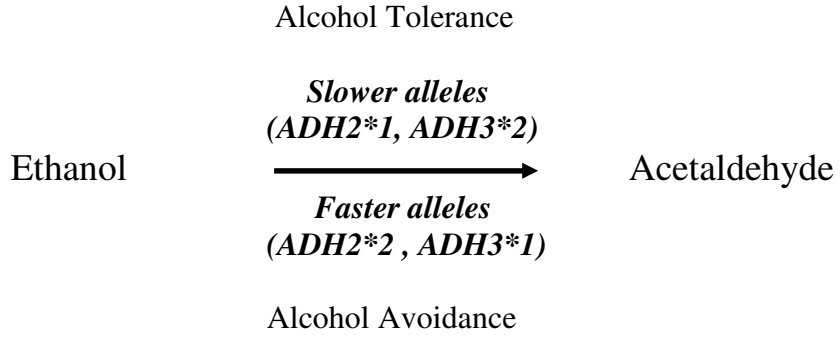

Figure 2.5. Metabolism of Ethanol is Influenced by Polymorphism in Alcohol Dehydrogenases (ADH) (Source: Roberts et al. 2004, Matsuo et al. 2007).

2.6.2. ALDH2 Gene Polymorphism and Alcoholism

Studies about ALDH gene polymorphisms concluded that the allele of ALDH2.2 is likely to play a protective role against excessive alcohol consumption (Figure 2.6.). By influencing drinking behavior in nonalcoholic individuals, ALDH2.2 may contribute to susceptibility for alcohol dependence. However, alcoholics with this inactive allele may be at a greater risk of advanced alcoholic liver disease (Vidal et al. 2004). According to the main hypothesis about the mechanism underlying this association, the isoenzyme encoded by the ALDH2.2 leads to impaired conversion of acetaldehyde to acetate (inactive allele) (Li et al. 2001, Roberts et al. 2004).

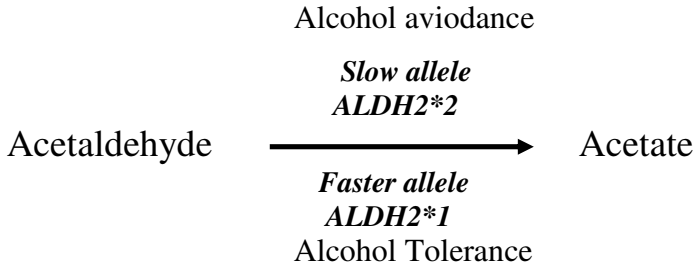

Figure 2.6. Metabolism of Ethanol is Influenced by Polymorphism in Aldehyde Dehydrogenase (ALDH) (Source: Roberts et al. 2004, Matsuo et al. 2007).

Association between reduced alcohol consumption or reduced risk of alcohol dependence and the ADH2.2 variant allele has recently been found in other ethnic groups that did not carry the ALDH2.2 allele. This is consistent with the finding that ADH2 affects susceptibility to alcohol dependence independent of ALDH2 (Li et al. 2001).

2.7. ADH2, ADH3 and ALDH2 Genes Polymorphism in the World Populations

The set of alcohol-metabolizing enzymes has considerable genetic and functional complexity. The relationship between some alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) genes and alcohol dependence have long been studied in many populations, but not comprehensively (Luo et al. 2006).

Molecular genetic studies concluded that there are differences in frequency of the ADH2, ADH3, ALDH2 alleles in different ethnic/racial populations (Vidal et al. 2004).

Table 2.3 shows the allele prevelances of polymorphic ADH2, ADH3 and ALDH2 genes in different ethnic groups.

Table 2.3. Allele Prevelances of Polymorphic Alcohol Metabolizing Genes in Different Ethnic Groups.

Allel type	Ethnic group	References
ADH2*1	• Predominate in most of world population (White and Black) (90%)	Chinnaswamy et al. 2005
ADH2*2	• Predominant in East Asian population (70%) • General White populations (0-10%) • European population (0-5.7%) • Spanish (7.8%) • French, American populations (2.3%) • German population (1.2%)	Chinnaswamy et al. 2005 Vidal et al. 2004 Borras et al. 2000 Li et al. 2001 Cichoz et al. 2007
ADH2*3	• African origins (20%)	Chinnaswamy et al. 2005
ADH3*1	• Predominates in black and East Asian populations (90%) • Caucasians (50-60%)	Li et al. 2001 Borras et al. 2000 Cichoz et al. 2007 Equal frequency in white populations
ADH3*2		
ALDH2*1	• Predominant allele in populations of White origin	Borras et al. 2000 Vidal et al. 2004 Roberts et al. 2004 Luo et al. 2006
ALDH2*2	• Prevalent in Asian populations • Rare in Caucasians	

CHAPTER 3

SINGLE NUCLEOTIDE POLYMORPHISM AND DETERMINATION TECHNIQUES

3.1. Single Nucleotide Polymorphism (SNP)

Polymorphisms can be defined as DNA sequence variations changing from individual to individual. In Single Nucleotide Polymorphism, a single nucleotide in the genome sequence is changed. It must occur in at least 1% of the population. It means that more than 99% of human DNA sequences are the same in the population. It is estimated that there are approximately three million SNPs in the human genome.

Although many SNPs have not any impact on cell function, they can play a significant role on response of people to diseases also drugs. So, currently SNP is the popular research component for biochemistry, medicine and pharmachemistry (Souvenir et al. 2003, WEB_1, 2007).

There are various techniques for finding SNP genotyping.

3.2. Methods for Genotyping SNP

Sequence-specific detection of SNPs (allelic discrimination) is divided into four general mechanisms.

3.2.1. Hybridization

With the hybridization approach, two allele specific probes are designed to hybridize to the target sequence only when they match perfectly (Figure 3.1.). Under the optimized assay conditions, the one-base mismatch sufficiently destabilizes the hybridization to prevent the allelic probe from annealing to the target sequence. Since no enzymes are involved in allelic discrimination, hybridization is the simplest mechanism for genotyping.

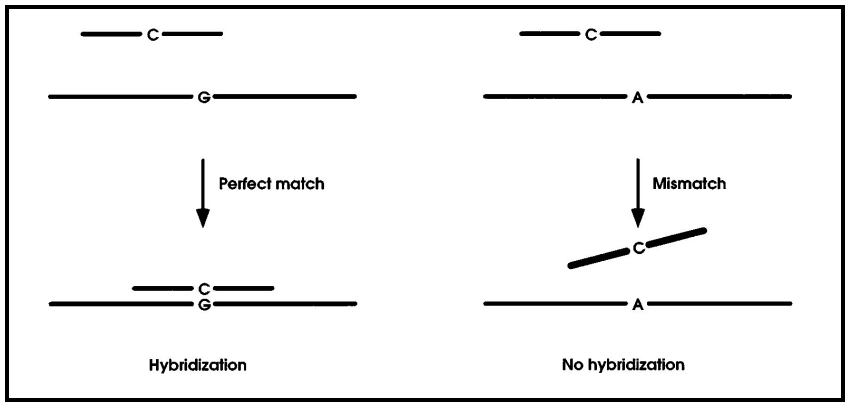

Figure 3.1. Allele–Specific Hybridization.
(Source: Kwok et al. 2001)

3.2.2. Primer Extension

Primer extension is highly flexible and requires the smallest number of primers/probes. There are numerous variations in the primer extension approach that are based on the ability of DNA polymerase to incorporate specific deoxyribonucleosides complementary to the sequence of the template DNA. They can be grouped into two categories (Kwok et al. 2001).

First is a sequencing (allele-specific nucleotide incorporation) approach where the identity of polymorphic base in the target DNA is determined (Figure 3.2.). Second is an allele-specific PCR approach where the DNA polymerase is used to amplify the target DNA only if the PCR primers are perfectly complementary to the target DNA sequence (Figure 3.3.).

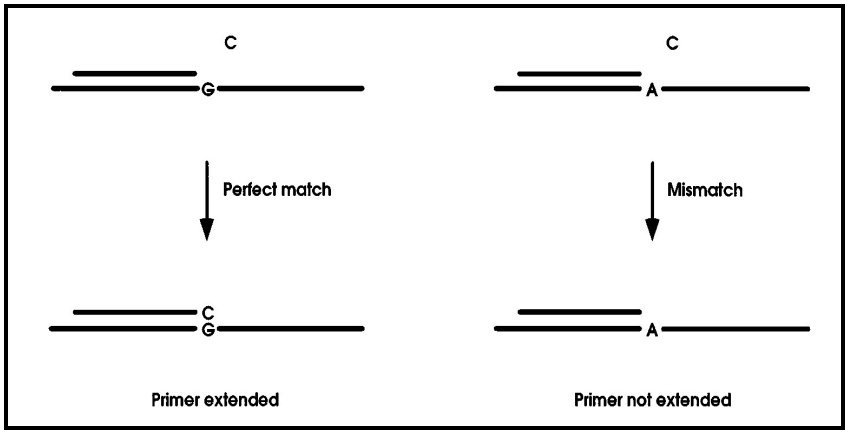

Figure 3.2. Allele-Specific Nucleotide Incorporation.
(Source: Kwok et al. 2001)

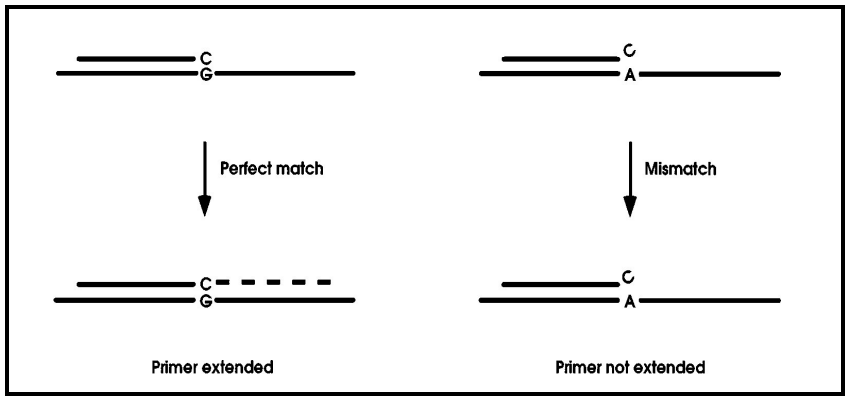

Figure 3.3. Allele-Specific Primer Extension.
(Source: Kwok et al. 2001)

3.2.3. Ligation

DNA ligase is highly specific in repairing gaps in the DNA molecule. When two adjacent oligonucleotides are annealed to a DNA template, they are ligated together only if the oligonucleotides perfectly match the template at the junction (Kwok et al. 2001).

Allele-specific oligonucleotides can, therefore, interrogate the nature of base at the polymorphic site. One can infer the allele(s) present in the target DNA by determining whether ligation has occurred (Figure 3.4.).

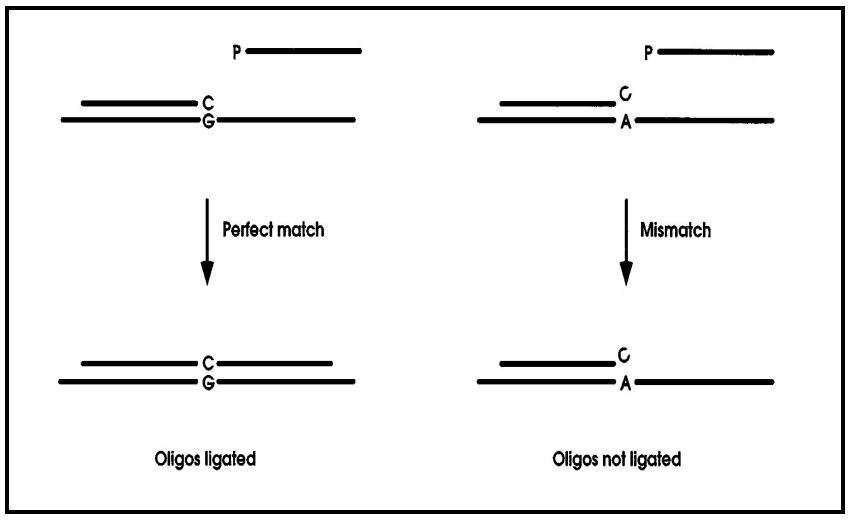

Figure 3.4. Allele-Specific Oligonucleotide Ligation.
(Source: Kwok et al. 2001)

3.2.4. Invasive Cleavage

Structure-specific enzymes cleave a complex formed by the hybridization of over-lapping oligonucleotide probes (Kwok et al. 2001).

When probes are designed such that the polymorphic site is at the point of overlap, the correct overlapping structure is formed only with the allele-specific probe but not with the probe with a one base mismatch (Figure 3.5.).

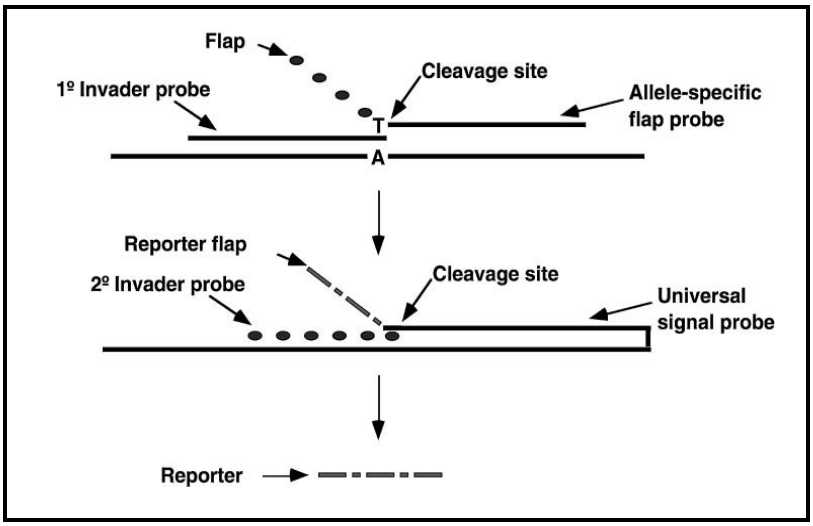

Figure 3.5. Invader Assay.
(Source: Kwok et al. 2001)

3.3. SNP Detection Mechanisms

Detection of a positive allelic discrimination reaction is done by monitoring the light emitted by the products, measuring the mass of the product, or detecting a change in the electrical property when the products are formed. The commonly used methods for genotyping are Luminescence Detection, Fluoresence Detection, Flourescence polarization, Flourescence Resonance Energy Transfer, Mass spectrometry and Electrical Detection (Kwok et al. 2001).

3.4 Principles of SNP Genotyping Methods Used in This Study

3.4.1. Polymerase Chain Reaction (PCR)

Polymerase chain reaction is a common method to create copies of specific fragments of DNA. PCR rapidly amplifies a single DNA molecule into many billions of molecules. A sample of genomic DNA can be used as the starting material for the PCR.

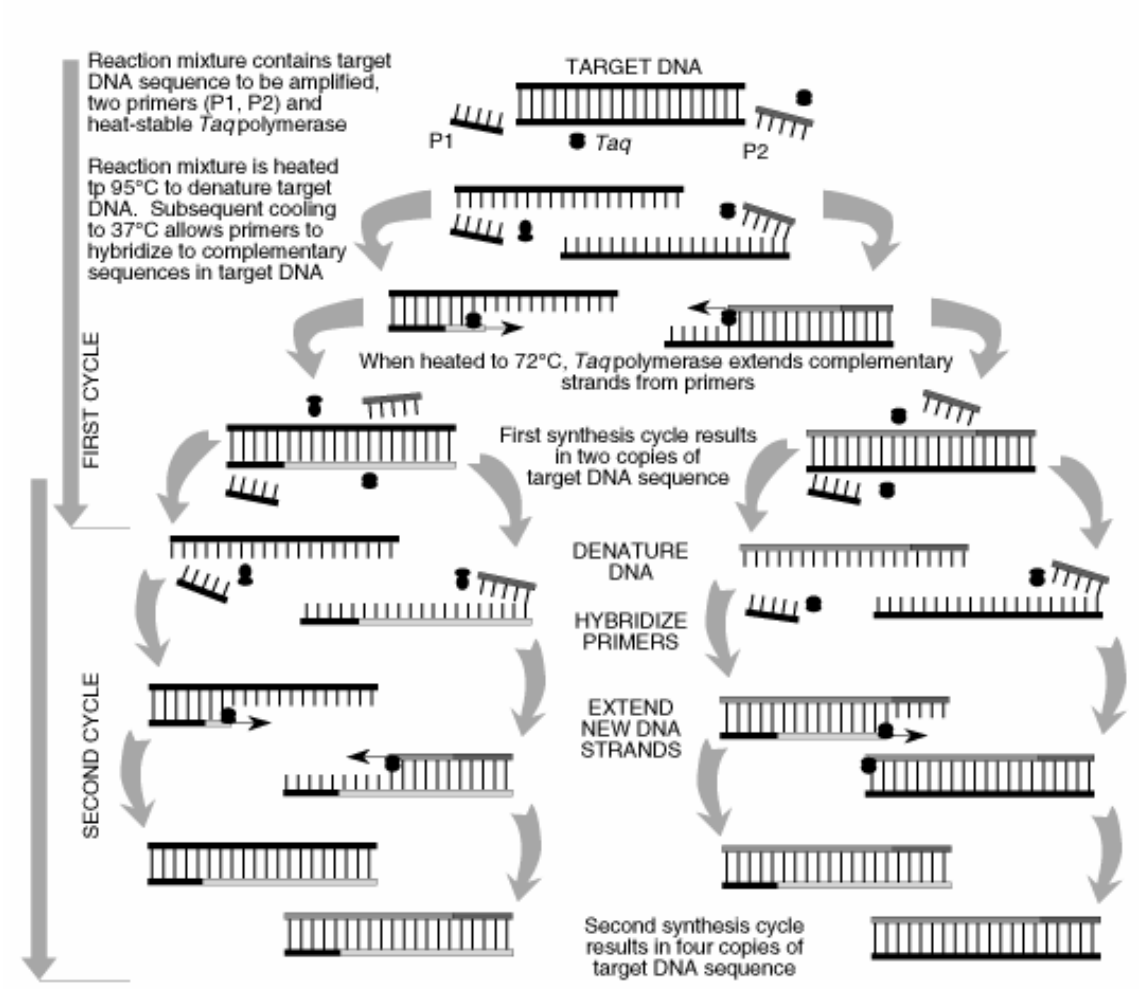

Figure 3.6. Polymerase Chain Reaction.
(Source: WEB_8, 2007)

In addition to target DNA a PCR reaction contains several other ingredients. These include free nucleotides, DNA primers and the enzyme Taq polymerase. These primers are typically about 20 nucleotides long and are complementary in sequence to the ends of the target DNA. Taq polymerase is derived from hot springs bacteria and

can tolerate the intense heat of a PCR reaction. A PCR reaction lasts several hours and typically consists of 20 to 35 repeating cycles.

A cycle begins by heating the reaction mixture to 95^0C. The heat denaturates the DNA by breaking the hydrogen bonds holding the strands together. After denaturating the DNA, the temperature is reduced to around 60^0C so that the primers can form hydrogen bonds, or anneal with their complementary sequences in the target DNA. In the next phase, the temperature is raised to 72^0C. Taq Polymerase function at this temperature is to give a start for polymerization, adding nucleotides to the 3' end of each primer attached to a DNA strand.

After one completed cycle, there are two-doubled stranded copies of the target DNA. The PCR reaction mixture contains many copies of the primers and an abundant supply of nucleotides to perform many addition cycles. As the number of cycles increases, the products consist of a greater proportion of with just the target DNA.

3.4.1.1. Multiplex PCR

Multiplex PCR is a variant of PCR in which two or more loci are simultaneously amplified in the same reaction. In other words, more than one target sequence can be amplified by including more than one pair of primers in the reaction (Figure 3.7.). Multiplex PCR has the potential to produce considerable savings of time and effort within the laboratory without compromising test utility. It is firstly described in 1988. This method has been successfully applied in many areas of nucleic acid diagnostics, including gene deletion analysis, mutation and polymorphism analysis, quantitative analysis, and RNA detection (Henegariu et al. 1999, Elfinro et al. 2000).

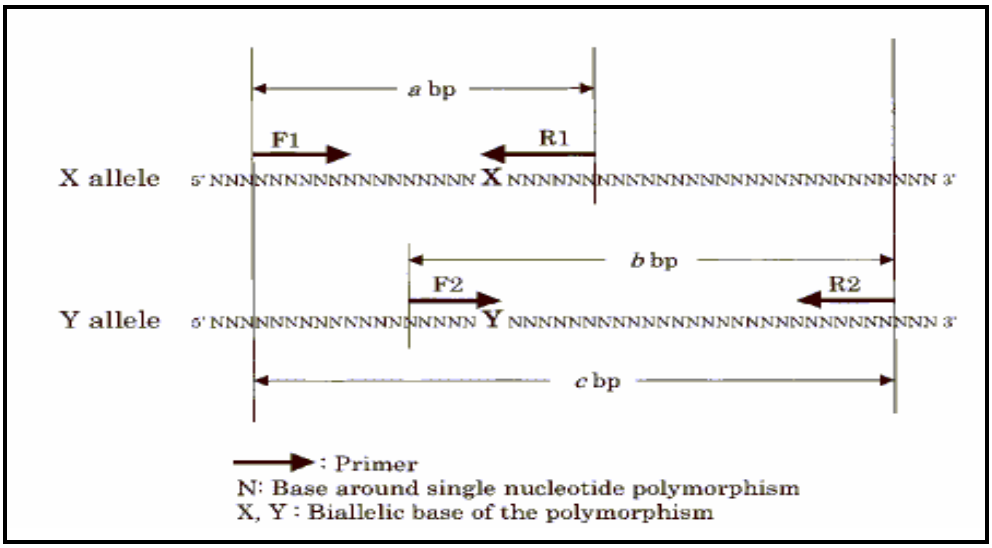

Figure 3.7. Basis of Polymerase Chain Reaction with Confronting to Two Pair-Primers. (Source: Tamakoshi et al. 2003)

3.4.1.2 Allele Specific Primer Extension

The target region is amplified by PCR followed by a single base sequencing reaction using a primer that anneals one base shy of the polymorphic site. In Figure 3.8., an application of allele specific primer extension method examined in detail.

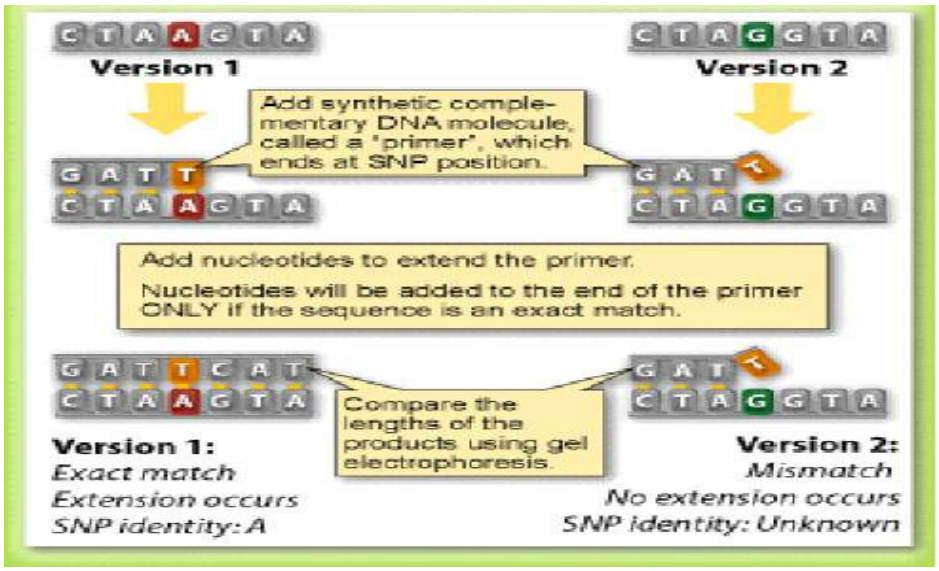

Figure 3.8. An Application for Allele Specific Primer Extension.

3.4.1.3. Restriction Fragment Length Polymorphism (RFLP)

Restriction Fragment Length Polymorphism (RFLP) is a technique in which organisms may be differentiated by analysis of patterns derived from cleavage of their DNA. If two organisms differ in the distance between sites of cleavage of a particular restriction endonuclease, the length of the fragments produced will differ when the DNA is digested with a restriction enzyme. The similarity of the patterns generated can be used to differentiate species from one another.

Restriction endonucleases are used to cut DNA fragment at specific sequences. If molecules differ in nucleotide sequence, fragments of different sizes may be generated. Sample DNA is digested with one or more RE's and resulting fragments are separated according to molecular size using gel electrophoresis (Figure 3.9.). Differences in fragment length result from base substitutions, additions, deletions or sequence rearrangements within RE recognition sequences (WEB_9, 2007).

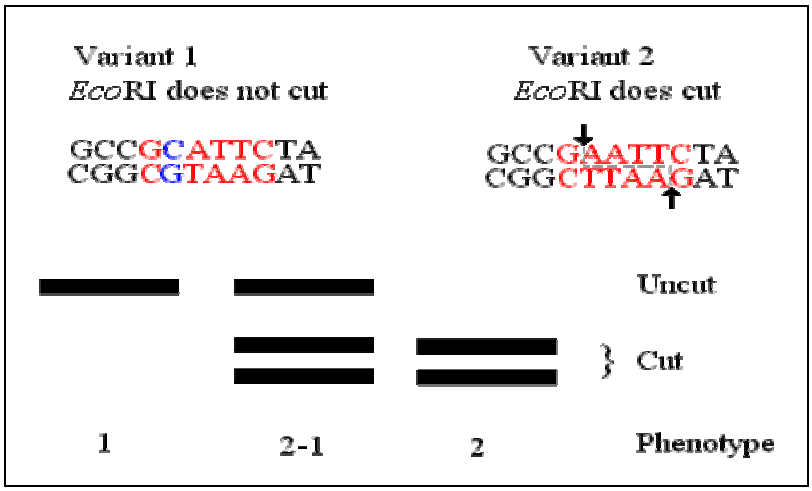

Figure 3.9. An Application for RFLP.
(Source: WEB_11, 2007)

Restriction endonucleases enzymes that cleave DNA molecules at specific nucleotide sequences depending on the particular enzyme used. Enzyme recognition sites are usually 4 to 6 base pairs in length. Restriction enzymes are isolated from a wide variety of bacteria where they appear to serve a host-defense role. In Figure 3.10., recognition regions for some restriction enzymes are shown.

Enzyme	Recognition Sequence	Enzyme	Recognition Sequence
*Alu*I	AG↓CT	*Hpa*II	C↓CGG
*Bal*I	TGG↓CCA	*Mbo*I	↓GATC
*Fnu*DII	CG↓CG	*Not*I	GC↓GGCCGC
*Hea*III	GG↓CC	*Sac*I	GAGCT↓C

Figure 3.10. Recognition Sites/Sequences for Common Restriction Enzymes.
(Source: WEB_10, 2007)

3.4.2. SNP Detection by Agarose Gel Electrophoresis

Agarose gel electrophoresis is the easisest and most common electrochemical separation process in which molecules, such as proteins, or DNA fragments, are made to move through an agarose gel, under the an electric current. The process consists of separating molecules on the basis of their molecular size and/or pH, by creating their differing rates of migration through the gel.

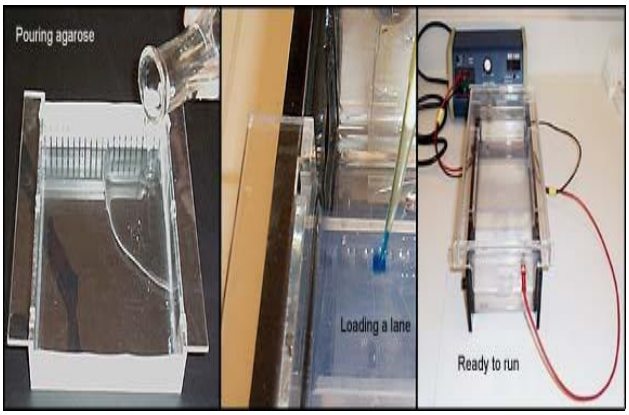

Figure 3.11. Agarose Gel Electrophoresis Equipments.
(Source: WEB_3, 2007)

In the case of DNA we can separate the molecules based on their size. DNA has a negative charge in solution, so it will migrate to the positive electrode in an electric field. In agarose gel electrophoresis the DNA is forced to move through a sieve of molecular proportions that is made of agarose. The end result is that large pieces of DNA move slower than small pieces of DNA.

The purpose of the gel is to look for the DNA, to quantify it or to isolate a particular band. The DNA is visualised in the gel by addition of ethidium bromide. This binds strongly to DNA by intercalating between the bases and is fluorescent. In other words, it absorbs invisible UV light and transmits the energy as visible orange light (WEB_3, WEB_4, 2007). In Figure 3.12., some examples of PCR Products are shown on agarose gel.

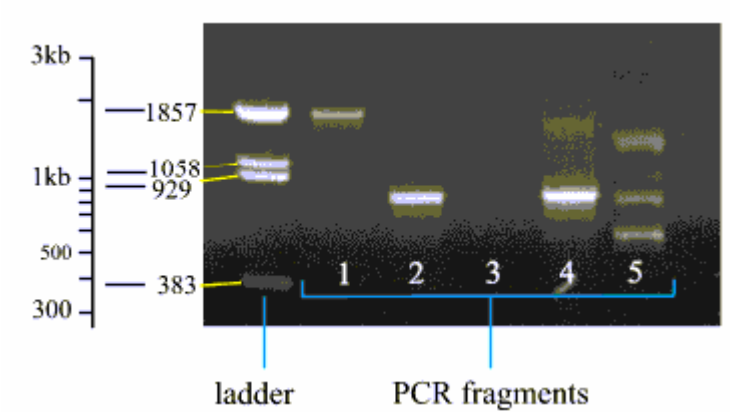

Figure 3.12. Verification of PCR Products on Agarose Gel.
(Source: WEB_12, 2007)

CHAPTER 4

MATERIALS AND METHODS

The methods used for determining ALDH2, ADH3 and ADH2 genotypes are shown in Figure 4.1.

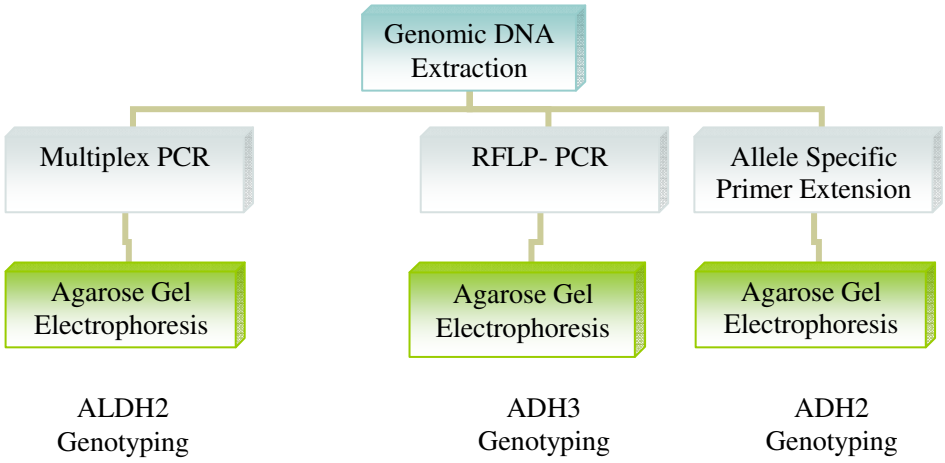

Figure 4.1. SNP Genotyping Methods Used in the Study.

4.1. Genomic DNA Extraction

200 µl sterile blood samples were collected from the subjects in EDTA-coated tubes and stored at +4 ^{0}C for DNA analysis. Genomic DNA extraction from the whole blood was done by using Invisorb Spin Blood Mini Genomic DNA Purification Kit. In Figure 4.2., the typical genomic DNA extraction steps are shown.

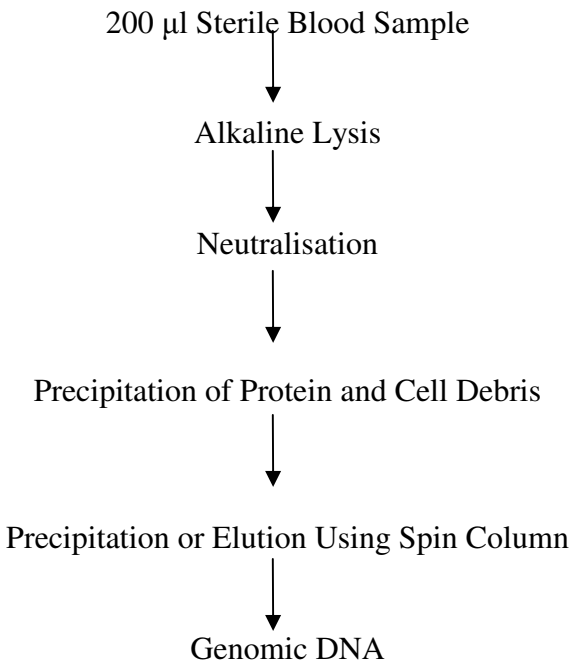

Figure 4.2. The Steps of Genomic DNA Extraction.

200 µl samples of whole blood cells yield 3-10 µg of DNA. Obtained genomic DNA samples were stored at -20 ^{0}C.

4.2. ADH3 Polymorphism by RFLP-PCR

4.2.1. ADH3 Amplification

ADH3 Amplification was carried out by using Gene Amp PCR System 9700 (Applied Biosystems)

PCR reaction reagents

1. 30-100 ng Genomic DNA
2. 0.18 mM dNTPs
3. 10 pmol of each primer
4. 0.5 Units of Hot Start Taq DNA polymerase
5. 2.5 µl x 10 TBE Buffer including 25 mM $MgCl_2$

Total PCR volume= 25 µl = 20 µl PCR mix (dNTPs+ primers+ Hot Start Taq DNA polymerase+ TBE Buffer with $MgCl_2$) +5 µl Genomic DNA

Hot Start Taq DNA polymerase was provided by Bioron Enzyme Company.

Used primers

PCR Primers were provided by MWG Biotech Primer&Probe design service.

Forward primer: 5'GCTTTAAGAGTAAATAATCTGTCCCC3'

Reverse primer: 5'AATCTACCTCTTTCCAGAGC 3'

(Vidal F. et al. 2004)

ADH3 PCR Programme

Number of cycle was 35.

Temperature (^{0}C)	**Time (sec)**
94	300
94	60
57	45
63	60
63	300
4	timeless

4.2.2. RFLP Application for ADH3

For allele detection, aliquots of amplified DNA products were digested with SspI (restriction enzyme) at 37^{0}C for 16 hours (overnight). SspI was provided by Bioron Enzyme Company.

SspI Recognition Site (cutting site) is shown below.

5'…AAT*ATT...3'

3'…TTA*TAA...5'

4.2.3. Evaluation of ADH3 Genotypes on Agarose Gel

Digestion products were run on 2% high resolution agarose gel and stained with ethidium bromide.

The genotypes identified were named according to the presence or absence of the enzyme restriction sites. So, G/G= *1/*1, G/A=*1/*2, A/A= *2/*2 are homozygotes for the absence of site (146 bp), heterozygotes (63-83-146 bp), and homozygotes for the presence of site (63-83 bp) (Vidal F. et al. 2004). In Figure 4.3., ADH3 genotypes are evaluated on agarose gel electrophoresis.

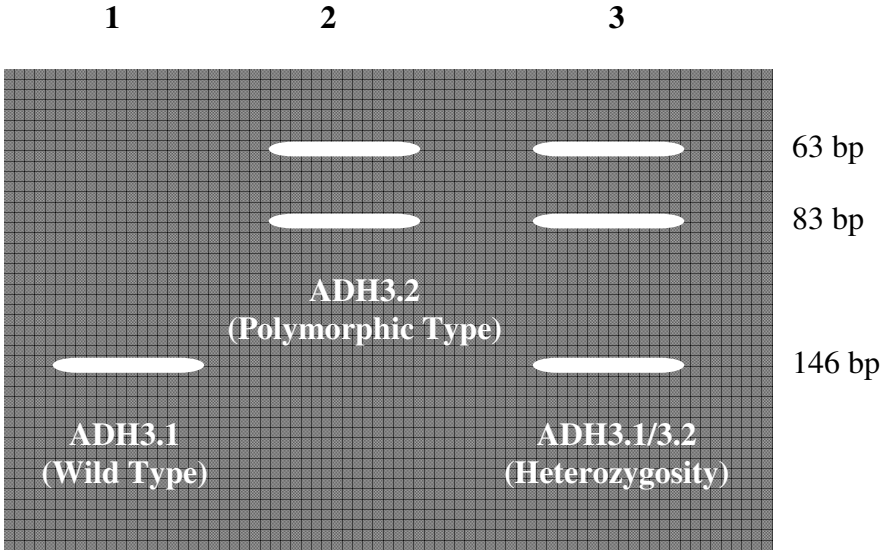

Figure 4.3. ADH3 Genotypes on Agarose Gel Electrophoresis.

4.3. ADH2 Polymorphism by Allele Specific Primer Extension

ADH2 Amplification was carried out by using Gene Amp PCR System 9700 (Applied Biosystems).

PCR reaction reagents

1. 30-100 ng Genomic DNA
2. 0.18 mM dNTPs
3. 10 pmol of each primer
4. 0.5 Units of Hot Start Taq DNA polymerase

5. 2.5 µl x 10 TBE Buffer including 25 mM $MgCl_2$

Total PCR volume= 25 µl = 20 µl PCR mix (dNTPs+ primers+ Hot Start Taq DNA polymerase+ TBE Buffer with $MgCl_2$) +5 µl Genomic DNA= 25 µl

Hot Start Taq DNA polymerase was provided by Bioron Enzyme Company.

Used primers

ADH2 gene located in 4q22 has a functional polymorphism Arg47His.

Two pairs of allele specific primers were used for ADH2 genotyping.

- For 47 Arg allele: ADH 2.1, 538 bp.

Forward Primer: 5'TCTGTAGATGGT GGCTGTAGGAATCTGACG 3'

This primer is Arginine specific (CGC).

The third base from 3' end is changed from T→A. This primer binds on Exon III.

Reverse Primer: 5'TACTTTTTTTCCCTCCTCCCGTTTCTACTTCTA3'

This is sequence specific primer binding on Intron III.

- For 47 His allele: ADH 2.2, 538 bp.

Forward Primer: 5'TCTGTAGATGGTGGCTGTAGGAATCTGCCA3'

This primer is Histidine specific (CAC).

The third base from 3' end is changed from T→C. This binds on Exon III.

Reverse Primer: 5' TACTTTTTTTCCCTCCTCCCGTTTCTACTTCTA3'

This is sequence specific primer binding on Intron III.

(Chinnaswamy et al. 2005).

PCR Primers were provided by MWG Biotech Company.

ADH2 PCR Programme

Number of cycle was 30.

Temperature (^{0}C)	Time (sec)
94	300
94	30
65	30
72	30
72	300
4	timeless

4.3.1. Evaluation of ADH2 Genotypes on Agarose Gel

In Figure 4.4., ADH3 genotypes are evaluated on agarose gel electrophoresis (An application for Arginine specific primer extension).

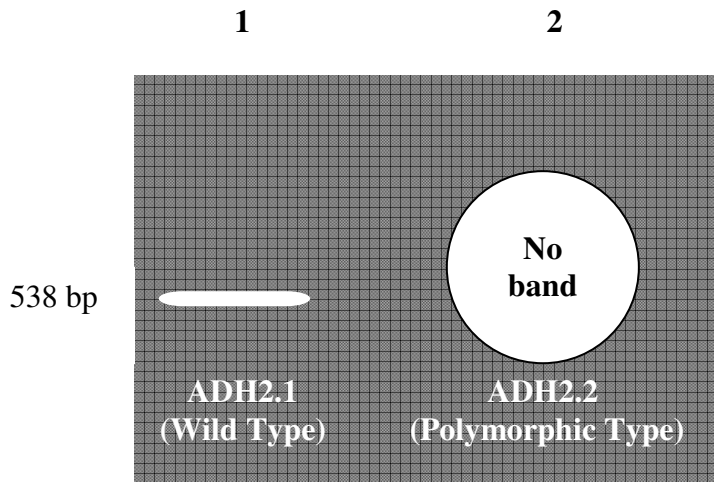

Figure 4.4. ADH2 PCR Products on Agarose Gel Electrophoresis.

4.4. ALDH2 Polymorphism by Multiplex PCR

ALDH2 gene located in 12q24.2 has a G -to-A polymorphism. (Glu487Lys)

- 487 Glu allele: ALDH 2.1, 119 bp
- 487 Lys allele: ALDH 2.2, 98 bp (polymorphic allele)

Base around Single Nucleotide Polymorphism represents 176 bp.

For ALDH2 amplification, Multiplex PCR was carried out by using GeneAmp PCR System 9700 (Applied Biosystems).

Used primers

Four primers (two primer pairs) were used for allele specific ALDH2 amplification. These PCR Primers were provided by MWG Biotech Company.

Forward primer1: 5'TGCTATGATGTG TTTGGAGCC3'
Reverse primer 1: 5'CCCACACTCACAGTTTTCACTTC
Forward primer 2: 5'GGGCTG CAGGCATACACTA
Reverse primer 2: 5'GGCTCCGAGCCACCA3'
(Tamakoshi et al. 2003)

PCR reaction reagents

1. 30-100 ng Genomic DNA
2. 0.18 mM dNTPs
3. 10 pmol of each primer
4. 0.5 Units of Hot Start Taq DNA polymerase
5. 2.5 µl x 10 TBE Buffer including 25 mM $MgCl_2$

Total PCR volume= 25 µl= 20 µl mix (dNTPs+ primers+ Hot Start Taq DNA polymerase+ TBE Buffer with $MgCl_2$) +5 µl Genomic DNA

Hot Start Taq DNA polymerase was provided by Bioron Enzyme Company.

ALDH2 PCR Programme

Number of cycle was 30.

Temperature (^{0}C)	Time (sec)
95	600
95	60
63	60
72	60
72	300
4	timeless

4.4.1. Evaluation of ALDH2 Genotypes Agarose Gel

In Figure 4.5., ALDH2 genotypes are evaluated on agarose gel electrophoresis.

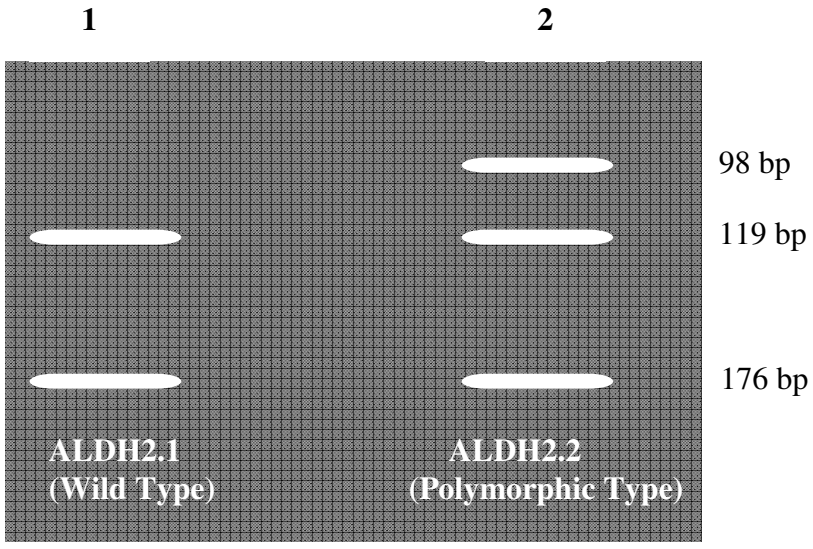

Figure 4.5. ALDH2 Genotypes on Agarose Gel Electrophoresis.

CHAPTER 5

RESULTS AND DISCUSSION

5.1. Results

5.1.1. Results for ADH 3 Genotyping

Obtained agarose gel electrophoresis screen for ADH3 genotypes of some alcoholic subjects is shown in Figure 5.1.

Lane

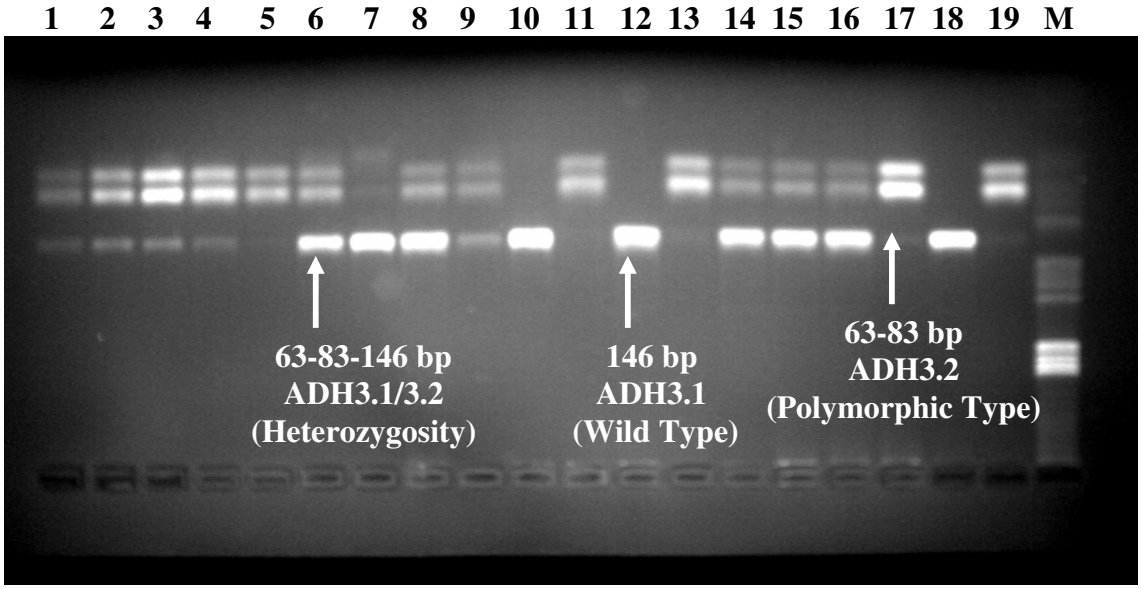

Figure 5.1. Screening of ADH3 Genotyping on Agarose Gel Electrophoresis.

Frequency of each genotype was determined for both alcoholics and control group. The significance of difference between two groups was determined by Fisher-Exact Test using GraphPad, Prism 4.0 statistical software. Table 5.1 shows the distribution of ADH3 genotypes for both alcoholics and control group.

Table 5.1. The Results for ADH3 Genotyping.

Gene	n*	Genotype			Allele (%)	
		ADH3.1 *1/*1(GG) (Ile/Ile)	**ADH3.1/3.2 *1/*2(GA) (Ile/Val)**	**ADH3.2 *2/*2(AA) (Val/Val)**	***1**	***2**
ADH3 (Alcoholic)	141	21 (14.89%)	75 (53.19%)	45 (31.9%)	41	59
ADH3 (Control)	80	5 (6.25%)	62(77.5%)	13 (16.25%)	45	55
p value (fisher-exact testing)		0.085	0.0003	0.0112	NS**	NS**

* Individual number
** No significant difference

As seen from Table 5.1, heterozygosity had the highest level of all ADH3 genotypes. Also, ADH3.2 genotype (polymorphic type) was more prevalent than ADH3.1 genotype (wild type). Distribution of ADH3 genotypes was different in alcoholics and controls. Slow metabolizing genotype, ADH3.2 was more prevalent in alcoholics. Allele frequencies approximately had homogenous distribution for both groups. On the other hand, there was no significant difference between alcoholics and control groups in the aspect of allele frequencies.

5.1.2. Results for ADH 2 Genotyping

Obtained agarose gel electrophoresis screen for ADH2 genotypes of some alcoholic subjects is shown in Figure 5.2.

Lane

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 M

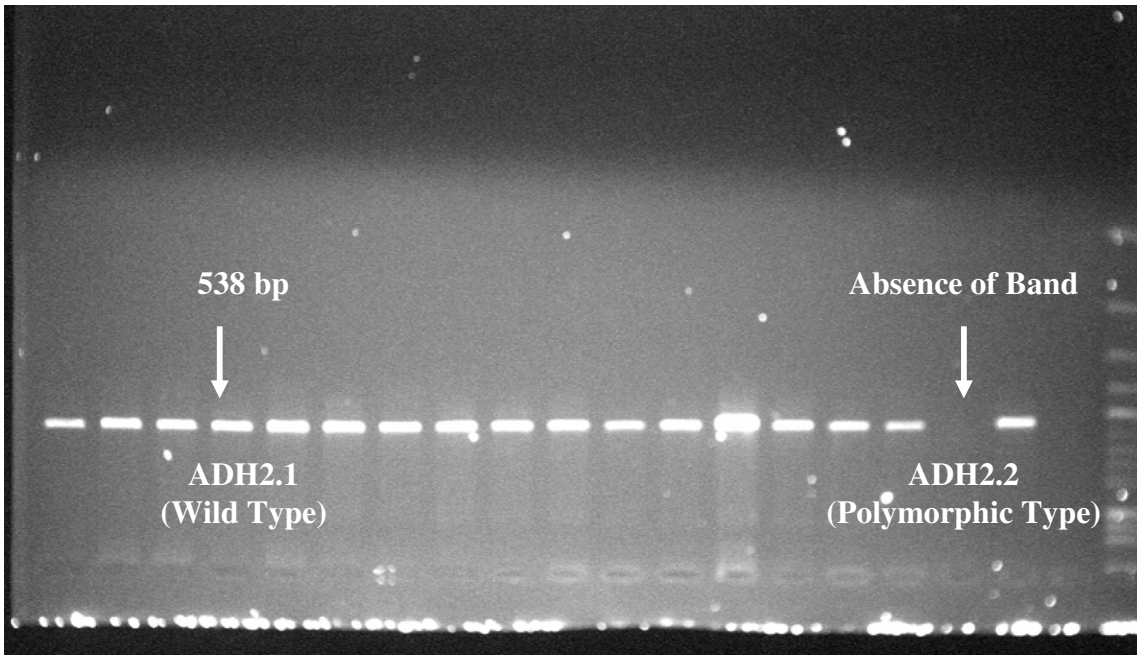

Figure 5.2. Screening of ADH2 Genotyping on Agarose Gel Electrophoresis.

Frequency of each genotype was determined for both patient and control group. The significance of difference between two groups was determined by Fisher-Exact Test using GraphPad, Prism 4.0 statistical software. Table 5.2 shows the distribution of ADH2 genotypes for both alcoholics and control group.

Table 5.2. The Results for ADH 2 Genotyping.

Gene	**n***	**Genotype**			**Allele(%)**	
		ADH2.1 *1/*1(GG) (Arg/Arg)	**ADH2.2 *2/*2(AA) (His/His)**	**ADH2.1/2.2 *1/*2(GA) (Arg/His)**	***1**	***2**
ADH2 (Alcoholic)	156	145(93.0%)	1(0.60%)	10(6.40%)	96	4
ADH2(Control)	81	80(98.70%)	0.0(0%)	1(1.30%)	99	1
p value (fisher- exact testing)		0.0631	1.0			

* Individual number

As seen from Table 5.2, ADH2.1 genotype (wild type) was more considerably higher than ADH2.2 genotype (polymorphic type) for both alcoholics and control

group. There was no significant difference between two groups for all ADH2 genotypes. Also, frequency of allele *1 was considerably higher than allele *2.

5.1.3. Results for ALDH 2 Genotyping

Obtained agarose gel electrophoresis screen for ALDH2 genotypes of some alcoholic subjects is shown in Figure 5.3.

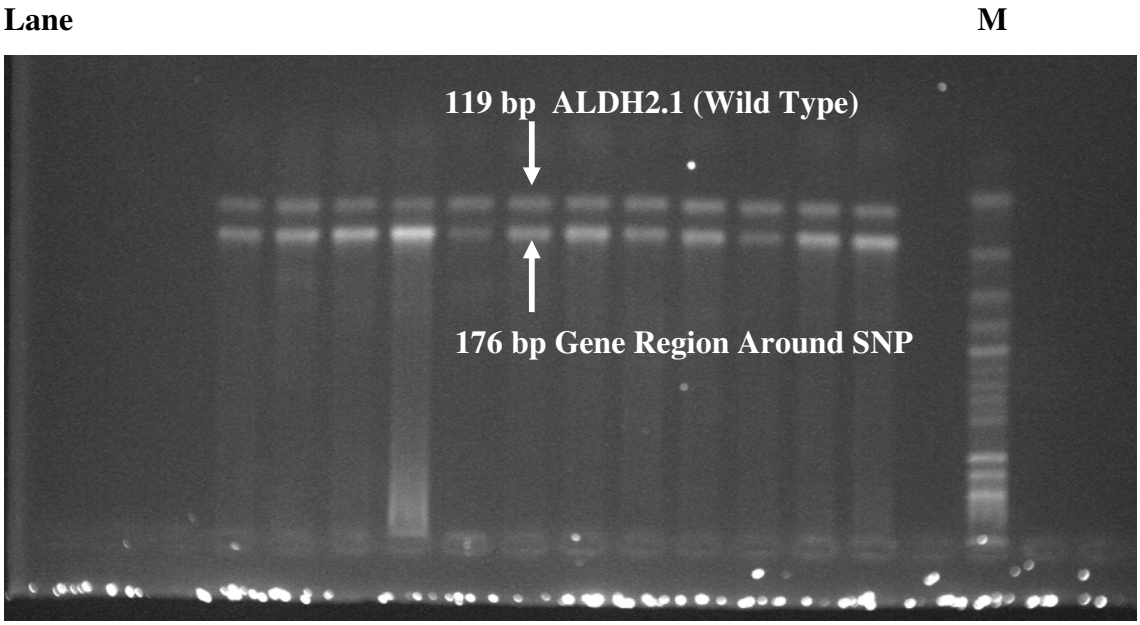

Figure 5.3. Screening of ALDH2 Genotyping on Agarose Gel Electrophoresis.

Frequency of each genotype was determined for both patient and control groups. Since the results for both groups are the same, no statistical test was applied. Table 5.3 shows the distribution of ALDH2 genotypes for both alcoholics and control group.

Table 5.3. The Results for ALDH 2 Genotyping.

Gene	n*	Genotype	
		ALDH2.1 *1/*1(GG) (Glu/Glu)	ALDH2.2 *2/*2(AA) (Lys/Lys)
ALDH2 (Alcoholics)	156	156 (100%)	0 (0.0%)
ALDH2(Control)	80	80 (100%)	0 (0.0%)

* Individual number

As seen from Table 5.3, all individuals in both alcoholics and control group had ALDH2.1 (wild type).

5.2. Discussion

It was shown that ADH3.1/3.2 (heterozygosity) is the most common genotype of all ADH3 genotypes. A higher frequency of ADH3.1/3.2 in Europeans has been demonstrated previously (Vidal et al. 2004). But, conversely, the differences we found in the ADH3.1/3.2 frequencies between examined groups were statistically significant. Many studies have concluded that the frequencies of allele *1 and allele *2 for ADH3 are equal in the white race. Our allele frequency distributions of ADH3 were consistent with previous reports in whites (Borras et. al 2000, Li et al. 2001, Cichoz et al. 2007). We found that frequency of ADH3.2 genotype is higher than ADH3.1 genotype in both alcoholics and controls. Since ADH3.2 is the slower allele for conversion of ethanol to acetaldehyde, it seems that Turkish people have tendency to have alcohol tolerance. Also, the higher frequency of ADH3.2 genotype in alcoholics comparing to the control group suggests that this genotype has a role in alcoholism. ADH3.1 genotype which is fasten the alcohol dehydrogenase activity was not different in alcoholics comparing with controls. Our findings were very similar to Borras et al. 2000. If all ADH3 genotypes are taken into consideration, the difference between control group and alcoholics was significant. So, in our population, ADH3 variation plays significant role in alcoholism.

Regarding ADH2, important differences are observed in allelic distribution between Oriental people and Caucasian races (Chinnaswamy et al. 2005). ADH2.1 is more prevalent in white populations (90%) in Oriental people (30%). Our ADH2 genotype distribution was consistent with previous reports in whites (Vidal et al. 2004, Chinnaswamy et al. 2005, Cichoz et al. 2007).

On the other hand, there was no considerable significant difference between alcoholics and control groups for ADH2.1. Like Bosron et al. 1986, Vidal et al. 1993 and Vidal et al. 2004, our results have suggested that ADH2.2 has not a role on alcoholism.

The frequency of ALDH2 alleles varies among populations (Cichoz et al. 2007). The ALDH2.2 allele rarely occurs in white populations. The investigations of the Caucasian race found an almost totally homozygotic character of ALDH2.1 (Borras et

al. 2000, Roberts et al. 2004, Vidal et al. 2004, Luo et al. 2006). We present similar observations in the study. No variations in ALDH alleles or genotypes were found. In the study, none of alcoholics and non-alcoholics examined presented ALDH2.2 inactive alleles. So, it can be concluded that ALDH2.2 inactive allele is not found in Turkish people like most of white races.

Table 5.4 shows the distribution of ADH2, ADH3 and ALDH2 genotypes in Caucasian race and Oriental people. As seen from Table 5.4, distribution of ADH2, ADH3 and ALDH2 genotypes in Caucasian race are strongly different from Oriental people. Our findings were very similar to Caucasian race as expected. Also, the prevalences of ADH3.2, ADH2.1 and ALDH2.1 which are slow alleles for ethanol metabolism have suggested that Turkish people have a considerable tendency to develop alcohol tolerance.

Table 5.4. Distributions of ADH2, ADH3 and ALDH2 Genotypes in Caucasian Race and Oriental people

Genotype	**Distribution in Caucasian Race (%)**	**Distribution in Oriental People (%)**	**Our Results (%)**
ADH3.1	50-60	90	45
ADH3.2	40-50	10	55
ADH2.1	90	30	96
ADH2.2	0-10	70	4
ALDH2.1	100	Rare	100
ALDH2.2	0	Prevalent	0

CHAPTER 6

CONCLUSION AND FUTURE PERSPECTIVE

If all the results are taken into consideration, it is inferred that Turkish people have the inherited variations of ADH and ALDH genes which do not protect them to have alcohol sensitivity and dependency. This case is consistent with the white race in the world including European people but not consistent with Oriental people as expected.

Finally, it can be said that ADH2, ADH3 and ALDH2 genes which involved in ethanol metabolism are the important contributors for increasing alcohol tolerance and they are strongly race dependent.

Further ADH and ALDH genes polymorphism research studies may be pioneers to identify new variants of these genes which protect Turkish people against alcoholism.

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