SciMedCentral

Journal of Substance Abuse & Alcoholism

Research Article

Determination of Genetic Polymorphism Taqia (Ankk1) Taqib (Drd2), -141c Ins/Del (Drd2) And 40 Bp Vntr (Slc6a3) in the Colombian Population and Evaluation of their Associations with Alcoholism

Tatiana Ximena Castro Matiz[1], Fabio Ancizar Aristizabal Gutierrez[2] and Mauricio Rey Buitrago[1]*

[1]*Department of Medicine, National University of Colombia, Colombia*
[2]*Department of Pharmacy, National University of Colombia, Colombia*

***Corresponding author**
Mauricio Rey Buitrago, Department of Medicine, National University of Colombia, Entrance Calle 53, building 426, Institute of Genetics. Bogota, Colombia, Tel. 571-316-5000; E-mail: mrey@unal.edu.co

Submitted: 06 July 2015

Accepted: 28 September 2015

Published: 30 September 2015

Keywords
- Alcohol Dependence
- Polymorphism
- Dopamine
- ANKK1
- DRD2

Abstract

Background: In the study of a multi factorial disease like alcoholism genetic component it is important because it can represent up to 50% of the influence, and there are significant ethnic differences among populations. This work is the first of its type for the Colombian population and aimed to analyze polymorphisms of the ANKK1 (TaqIA), DRD2 (TaqI B and -141C Ins/Del) and SLC6A3 (VNTR 40 bp) genes in a Colombian alcohol-dependent individuals to determine their associations with alcoholism.

Methods: Genotypes of these polymorphisms were determined by conventional PCR and PCR-RFLP in a population of 214 individuals with non-problematic alcohol use and individuals with problematic alcohol use. The DNA was obtained from peripheral white blood cells.

Results: Several hypotheses have suggested that people with problem drinking have decreased density and presynaptic dopamine reuptake problems due to disturbances caused by polymorphisms associated with the ANKK1, DRD2 and SLC6A3 genes. The gene and allelic frequencies of 214 individuals showed significant differences for allele - 141C Ins in individuals who had an AUDIT score of 14 to 19 and for the Taq IB allele in women with problematic alcohol consumption. A meta-analysis was done for association of polymorphism of DRD2 gene Taq1A with alcoholism. The results of this meta-analysis may add to the long list of studies that have yielded A1 allele association with alcoholism.

Conclusion: In the present study no association was found between polymorphism of DRD2 gene Taq1A with alcoholism in this Colombian population. It is also suggested that the Ins -141C allele was a critical factor in the harm phase of dependent alcohol consumption. Similarly, a significant difference for the allele IB TaqIB DRD2 gene polymorphism in the group of women with alcohol dependence was found.

INTRODUCTION

Excessive alcohol consumption is one of the public health problems of greatest magnitude relative to legal psychoactive drug use, and it is a global problem that endangers the individual development, family life and social life of a person. It is estimated that approximately 2.6 billion people worldwide consume alcohol occasionally, habitually, abusively or addictively; every year, 2.5 million people die from alcohol-related causes [1,2]. In Colombia, data from the PRADICAN (project-Anti-Illegal Drugs Program of the Andean Community), conducted in 2012, showed that 95.6% of college students reported having consumed alcohol at least once in their lives, 85% declaring recent use and 61.6% reporting regular consumption. At the same time, the AUDIT (Alcohol Use Disorders Identification Test) test was used to inquire about problem drinking, and the results showed that 75.2% of the surveyed population showed risky behavior, i.e., 3 out of 4 people could be considered as risky drinkers or detrimental users. Fourteen percent of the total population showed signs of dependence [3]. The frequent and excessive consumption of alcohol is related to more than 60 health conditions, ranging from those that are the result of excessive alcohol consumption during pregnancy and affect the fetus, to intentional injury, liver disease, cancer and neuropsychiatric conditions, including dependence on psychoactive drugs [4]. Alcohol dependence has a complex

heritage character and greater heterogeneity, in which multiple factors as psychological and genetic can be involved and in which the environment plays an important role in the development of the disorder, and the need for precise exposure conditions must be met. The genetic component can account for up to 50% of the influence, as demonstrated by twin studies, adoption studies and animal models [5]. The function of the dopaminergic pathways of the central nervous system (CNS) has become the focus of great interest because various changes in transmission are related, directly or indirectly, to severe CNS disorders, such as Parkinson's disease, psychotic disorders including schizophrenia and dependence on psychoactive drugs including alcohol [6]. The dopaminergic pathways involved in the development of addiction have two zones: ventral tegmental area (VTA) and nucleus accumbens (NAc). In the VTA, the cell bodies of these neurons send projections to brain regions involved in emotion, thought, memory, planning and executing behaviors. The NAc is involved in motivation, learning and the assessment of the motivational value of stimuli. Psychoactive substances increase dopamine release in the NAc, and it is believed that this release is important in reinforcing addictive behavior as part of a brain reward circuit that is associated with positive moods, such as pleasure, or euphoria events [7,8,9]. With the cloning of the gene for type 2 dopamine receptor (DRD2) were described several polymorphisms, two of which were associated with alcohol dependence: TaqIB (rs1079598 at 11:113296274) and -141C Ins/Del (rs1799732) causing decrease of dopamine in the reward system that can cause dysphoria, anhedonia and depression, or due to alcohol, it could improve a negative mood [8]. The TaqIB polymorphism was detected in the 5' extremity within the coding region but very close to the gene promoter region, adjacent to exon 2 [10]. The polymorphism is a change from a guanine to an adenine. Located within the coding region, it plays an important role in the function of the gene. The two alleles described for this polymorphism are B1 and B2. Some authors have associated the gene with substance abuse, while others have found no such association [11,12]. The DRD2 polymorphism -141C Ins/Del is located in the promoter region, approximately 250 kb from the 5' end of the gene. Alleles marked by the presence and absence of a cytosine at position -141 were described as DRD2-141C Ins and DRD2 -141C Del, respectively [13]. The deletion of a cytosine is associated with reduced transcriptional activity, compared to the insert. However, several studies have shown conflicting results revealed associations and no associations between the -141C allele and alcoholism [14,15,16].

Initially, the TaqIA (rs1800497 at 11:113270828) polymorphism was believed to be located on the DRD2 gene, consistent with a change from a cytosine to a thymine. It is currently considered a change detected by RFLP, located near the 3' end of exon 8 of the ANKK1 gene (Ankyrin repeat and Kinase domain containing 1). This change results in a mis sense substitution (E713K) in the eleventh repeat ANKK1 [17] that not alter the structural integrity of the protein, but it can affect the specificity of binding to the substrate; therefore, if replacement occurs, it could cause a change in the activity of ANKK1, which might provide an explanation for the association of the polymorphism with neuropsychiatric disorders, such as addiction. This substitution has been associated with increased enzymatic activity involved in dopamine biosynthesis in the striatum of L-dopa, [18]. Two alleles have been described for the polymorphism, A1 and A2, with A1 associated with a reduction in the D2 receptors in pre- and postsynaptic neurons [19-21].

The SLC6A3 (DAT1) is a protein member of the family of Na^+/Cl^- dependent transporters that modulates synaptic dopamine uptake in dopaminergic neurons. Free dopamine reuptake in the synapse is the primary mechanism of the modulation of dopaminergic function, and it is blocked by addictive substances such as alcohol, which explains the stimulatory effect of this drug on dopamine [22]. The SLC6A3 is present in the presynaptic membrane, carrying dopamine from the synaptic cleft into the cytosol [23,24]. It is also responsible for the dopamine recapture in both directions at the points of synapses, normally doing so at the nerve terminal. The VNTR polymorphism of 40 bp dopamine transporter has also been associated with addiction to alcohol. Being located at the 3' UTR end does not affect the configuration of the protein, but it does affect the stability and transcription of mRNA. The 40 bp can be repeated three to thirteen times, but nine and ten repetitions are the most common, and repetitions affect the expression of the transporter in the striatum [25]. In individuals heterozygous for 9/10 repeats, an average reduction of 22% of the carrier protein in the putamen was observed when compared with homozygous individuals; this finding might explain the relationship of the polymorphism with the severity of withdrawal symptoms and with other phenomena in the development of clinical disease [26].

It was decided to conduct a meta-analysis of the association of polymorphism of DRD2 gene Taq1A with alcoholism. In 1990, Blum et al. [27] established the first association of polymorphism of DRD2 gene Taq1A with alcoholism. This polymorphism has been one of the most studied seeking to replicate this first finding, but the results have consistently deferred regarding the association of the polymorphism with alcohol addiction. The reasons for obtaining these ambiguous results could arise from ancestral variation, phenotypic variation, sampling variation or some other source of heterogeneity.

Initially it took just a search for scientific articles in PubMed database using the terms "alcohol", "alcoholism", "DRD2", "dopamine" and "TaqIA". Once items are classified, they were analyzed in relation to the following criteria: studies that involved individuals of genders, ethnicity, age, and which allelic frequencies were expressed regarding the A1 and A2 alleles. Studies using data from only just alcoholics or individuals healthy participants were excluded. In addition, only studies reporting that the study population was in Hardy-Weinberg equilibrium were included. Studies that reported previously published data were excluded.

For each article reviewed the data taken into account were: author, year of publication, country of origin, ancestry, sample size, diagnostic criteria, and genotyping and allele frequencies. The descent was coded as European, East Asian, American and others.

Data were analyzed using the program Epidat version 3.1, which yields data heterogeneity, publication bias and sensitivity analysis of the frequencies reported in each of the studies

included in the meta-analysis. Statistical significance of data was p = ≤0.05.

A total of 20 studies published between 1990 and 2010 were identified through the search strategy considering the inclusion and exclusion criteria.

When performing the respective statistical analysis of the 20 items selected and included, the Dersimonian and Laird models for heterogeneity indicated there is no statistical evidence of heterogeneity (X^2 = 25.3999, d.f. 19, p = 0.1478 and 95% C.I.). When analyzing the Galbraith plot is evident that the studies are not homogeneous at 100%, demonstrating heterogeneity, with a clear influence of studies Blum [27], Coming [28], Berggren [29] and Goldman [30], of falling out of the confidence bands of the graphic.

The result obtained from the analysis of heterogeneity indicated that it is advisable to use the random effects model to obtain the overall estimate of effect. This result indicated that the A1 allele polymorphism Taq1A is a risk allele for people who carry on the development of addiction to alcohol compared to control individuals of the populations studied. The association test was statistically significant (OR = 1.2352, 95% CI 1.0717 to 1.4237).

With regard to publication bias, Begg´s test yields no significant results (Z = 1.8493, p = 0.0644). Of the 20 selected studies, only two points are observed outside the boundary lines of the "funnel". The Egger and Begg tests not shown significant differences (t = 1.6390, df = 18, p = 0.1186), therefore we cannot reject the hypothesis of no risk and it is unlikely that the results are misunderstandings to publication bias.

The sensitivity analysis performed with the selected studies demonstrates the robustness of this meta-analysis. When any of the included studies did not change the direction or the significance of the overall effect is eliminated.

The results of this meta-analysis based on the search for the association Taq1A A1 allele polymorphism of DRD2 dopamine receptor, showed significant results on the risk of this allele and alcoholism. The combination of all the selected studies showed an OR = 1.2352, indicating an increase of alcoholism in individuals carrying the A1 allele polymorphism Taq1A.

Statistical analyzes showed that the items are homogeneous; although it was observed in the corresponding plots some outdated or out of the cut lines points. The heterogeneity of these outdated studies may be attributed to study designs and methodologies, as well as sample sizes. For example the study of Beggren et al. [29] has the largest sample of all included studies (2348 individuals), while the study of Blum et al. [27] contains one of the smaller samples (70 individuals).

With regard to publication bias, evidence for the absence of bias was observed, this observation is important because the literature on the association Taq1A polymorphism and alcoholism can be examined with reasonable power by the large number of studies conducted and published worldwide.

The results of this meta-analysis may add to the long list of studies that have yielded A1 allele association with alcoholism.

MATERIALS AND METHODS

Patients

The sample size was calculated using the Epi Info™ program (matched case-control), depending on the allele frequencies reported in the literature and considering an alpha value of 0.05 and a confidence interval of 95%. To determine the frequency, the results of the study by Konishi, conducted in a Mexican-American population [31], was consulted. A comparative study was performed between two Colombian populations, who were classified according to the AUDIT instrument [32] into a control group (111 individuals with occasional consumption of alcohol) and a case group (103 individuals with problematic alcohol use). The control subjects were those individuals who obtained a score less than 7 on the AUDIT questionnaire, indicating no risky behavior in the consumption of alcohol. The case group consisted of individuals who scored higher than 13, indicating a high level of alcohol problems. All of the members of the subgroups analyzed were paired with their respective controls, considering the ages and sexes of the participant individuals in the case group. All of the subjects in this study provided informed consent for their participation.

Experimental procedures

The classification of polymorphisms in the ANKK1, DRD2 and genes was performed at the Laboratory of Human Genetics of the Institute of Genetics at the National University of Colombia. DNA was obtained from peripheral blood cells collected by a finger stick of the ring finger of the hand using a lancet. Samples (3-5 drops) of blood were collected on FTA cards. The DNA extraction process used Whatman reagent for a first washing of an indentation of the FTA paper impregnated with blood, followed by incubation at room temperature for 30 minutes, 2 or 3 washes with distilled water and a drying step for 1 h at 60 °C [33].

Genotyping of ANKK1 Taq IA

The identification of the polymorphism was performed by the RFLP technique. PCR was performed to a final volume of 25 µL containing FTA paper Notch genomic DNA, 2.0 µL of Assay Buffer 10X, 1,25 µl of dNTPs mix, 1,5 µl of MgCl2, 1 unit (U) of Taq polymerase, 1,0 µl of each primer (0.1 µM) FW: 5'- GAGACAGGGTTTTGCCATGT-3´,RV: 5'-CGGTCCAGAGGAGCACCT-3' and water to complete the final volume. The thermal cycling conditions were: 1 cycle (95 ° C for 5 min), 30 cycles (95 ° C for 30 s, 57.4 ° C for 30 s, 72 ° C 30 s), and 1 cycle at 72 ° C for 5 min. The amplicons were separated on 1.4% agarose gels. Using a molecular weight marker (100 bp ladder) 425 bp bands were observed. The PCR products were digested with TaqI enzyme under the following conditions: the amplified 6UL was obtained, and 6.2 µL of molecular biology grade water, 1.5 µL of 10X assay buffer, 0.3 µL of BSA and 1.0 µL of TaqI enzyme were added. Digestion was conducted for 2 hours at 65 ° C. The fragments were visualized on 1.4% agarose gel. For the A1 (C to T change) allele, the observed restriction site of a 425 pb band was lost. In presence of the WT allele, the A2 cleavage site for this enzyme (TCGA) yielded two bands: one band of 141 pb and the other of 284 pb. One of the products was sequenced to confirm the individual's identity, with a T was observed at position 254.

Genotyping DRD2 Taq IB

The conditions of the PCR reaction were identical to the above with the following primers: FW: 5'–GATACCCACTTCAGGAAGTC-3', RV: 5'-GATGTGTAGGAATTAGCCAGG-'3 (0.1 μM) and water for the final volume. The thermal cycling conditions were: 1 cycle (95 ° C for 5 min), 30 cycles (30s 95 ° C, 57.4 ° C for 30 s, 72 ° C for 30 s), and 1 cycle 72 ° C for 5 min. The amplicons were separated on 1.4% agarose gels, and a molecular weight marker was used (100bp ladder). One band of 459 pb was observed. The PCR products were digested with TaqI enzyme under the following conditions: the amplified 6UL was obtained, and 6.2 μL of molecular biology grade water, 1.5 μL of 10X assay buffer, 0.3 μL of BSA and 1.0 μL of TaqI enzyme were added. Digestion was performed for 2 hours at 65 ° C. The fragments were visualized on a 2% agarose gel. For the A1 allele (mutant), the observed restriction site with 459 bp band was lost. For A2, in the presence of the allele (WT), the cleavage site for this enzyme (TC/GA) resulted in two bands of 254 bp and 205 bp, respectively.

Genotyping DRD2-141C Ins/Del

The conditions of the PCR reaction were identical to the above with the following primers: FW: 5'- CAACCCTTGGCTTCTGAGTC-3' and RV: 5'- CCACCAAAGGAGCTGTACCT-'3 (0.1μM)). The thermal cycling conditions were: 1 cycle (95 ° C for 5 min), 30 cycles (95 ° C for 30 s, starting at 67 ° C and down 1 ° C per cycle 30 s, 72 ° C for 30 s), and 1 cycle 72 ° C for 5 min. The amplicons were separated on 1.4% agarose gels. A molecular weight marker was used (100 bp ladder), and a band of 215 pb was observed. The PCR products were digested with the BSNI (New England Biolab) enzyme under the following conditions: 6 μL were amplified and obtained, consisting of 5.6 μL of molecular biology grade water, 1.5 μL of assay buffer 10X, 1.0 μL of BSA and 0.6 μL of BStNI enzyme. Digestion was performed for 2 h at 60 ° C. The fragments were visualized on a 9% polyacrylamide gel. For the Ins allele (mutant) restriction site, the observed band with 215 pb was lost. In the presence of the allele, the product of cleavage site for this enzyme (CC/WGG) resulted in two bands of 117 pb and 38 pb, respectively.

Genotyping 40bp VNTR

genotyping VNTR amplification was performed by PCR to a final volume of 25 μl, containing approximately 200 ng of genomic DNA, 2.5 μl of 10X Assay Buffer, 1.25 μl of dNTPs mix, 1.5 μl of MgCl2, 1 U of Taq polymerase and 1.0 μl of each primer (0.1 μM) (FW: 5'-AAACCAGCTCAGGCTACTGC-3´ and RV: 5'-AGGCAGAGTGTGGTCTGCA-'3). The thermal cycling conditions were: 1 cycle (95 ° C for 5 min), 30 cycles (30s 95 ° C, 57.4 ° C for 30 s, 72 ° C for 30 s), and 1 cycle 72 ° C for 5 min. The amplicons were separated on 1.4% agarose gels. A molecular weight marker was used (100 bp ladder). To corroborate the results obtained, a sample from an individual was sequenced. **Statistical analysis**: To estimate the allelic and genotypic frequencies of the ANKK1, DRD2 and genes, the GENEPOP genetic data analysis program [34], was used. The relationships of genotype and allele frequencies were assessed using the chi square test and a P value

RESULTS AND DISCUSSION

By stratifying the total study population by age (214 individuals), we found that 11% of the individuals were 18-19 years old, 35.5% were 20-29 years old, 22% were 30-39 years old, 20% were 40-49 years old, 7.0% were 50-59 years old, and 4% were 60-67 years old; the population was concentration between 20 and 30 years old. Of the 103 alcohol-dependent individuals, 83 belonged to foundations or support groups. The other 131 participants were linked through the National University of Colombia, where invitations were extended to students, teachers, administrators, etc. Of the 103 alcohol-dependent individuals, 29 (28%) were women, and 74 (72%) were men. The difference between the numbers of women and men being different from the lower incidence of alcoholism in women could be attributed to women seeking help less often because of the stigma of society and their recognition of the danger posed by their addiction; for example, they could lose custody of their children. By stratifying men and women by age and sex, it was found that 11 of the 29 women were located in the age range of 20 to 29 years old, constituting 38% of the female population. In the men's group, it was found that, of the 74 men, 20 of them are in the range of 30 to 39 years old, corresponding to 27%. All 214 of the participants completed the AUDIT questionnaire as a tool for classification. By classifying alcohol-dependent individuals by age, sex, and AUDIT score, it was noted that, of the 103 subjects, 32 (31%) of them scored 14-19 (harmful use), and 71 (69%) scored 20-40 (physicochemical drinking problem, possible dependence). The gender distributions of the alcohol-dependent group and the group with occasional consumption showed that the number of alcohol-dependent individuals was higher in men than in women (Table 1). All of the participants completed a semi-structured questionnaire on consumer habits, health status and other questions to characterize the sample better. From the answers to the questionnaire, the following was found. Regarding family history, 21% of individuals with occasional alcohol consumption and 73% of the alcohol dependent respondents reported having a family member with alcoholism (P = 0.001), with both the father and uncles reported. Regarding use of other substances, 66% of individuals with alcohol dependence and 18% of casual alcohol consumers responded affirmatively, with significant differences between the two groups (P = 0.001). The most commonly used substances in the alcohol-dependent group were marijuana, powder and crack cocaine, while the group with occasional drinking reported marijuana consumption, followed by cigarettes. The age of onset of alcohol use was concentrated between 13 and 18 years old for both groups (68% of alcohol dependent subjects and 65% of occasional consumers of alcohol); 3% of alcohol-dependent individuals responded that the age of onset was between 7 and 9 years old. Eleven percent of alcohol-dependent individuals and 5% of individuals with occasional alcohol consumption reported having initiated drinking at 10-12 years old. For 5% of individuals with alcohol dependence and 10% of casual consumers, the onset age was 19-21 years old.

Gene and allelic frequencies

The allelic and genotypic frequencies of the TaqIA, TaqIB, -141C Ins/Del polymorphisms and 40 bp VNTR in alcohol-dependent subjects and subjects with occasional alcohol consumption are shown in Tables 2 and 3, with no significant differences in the frequencies of alleles and genotypes between the two groups when not stratified for any of the four

Table 1: Stratification of the sample of alcohol dependent population by alcohol-dependent AUDIT score, sex and age group.

Age	AUDIT Score				Total
	14-19		20-40		
	Male	Female	Male	Female	
18-19	2	4	1	2	10
20-29	14	2	13	9	38
30-39	4	1	16	3	24
40-49	3	1	12	5	21
50-59	0	0	6	1	7
60-67	0	1	2	0	3
Total	23	9	51	20	103

Table 2: Genotype and gene frequencies for TaqIA, TaqIB, -141C Ins/Del and 40 bp VNTR polymorphisms of the ANKK1 and DRD2 genes.

Gene polymorphism and group	N	Frequency Genotype % (N)			Gene frequency%	
		1/1	1/2	2/2	1	2
ANKK1-TAQIA						
Alcohol-dependent	103	18,5 (19)	37,9 (39)	44,7 (45)	37,4	62,6
Control	111	10,8 (12)	37,8 (42)	51,4 (57)	29,7	70,3
P value (<0.05)	X^2=4,93 P=0,08					
DRD2-TAQIB						
Alcohol-dependent	103	13,6 (14)	34,9 (36)	51,5 (53)	31,1	68,9
Control	111	16,2 (18)	26,1 (29)	57,6 (64)	29,3	70,7
P value (<0.05)	X^2=0 P=1,0					
DRD2 -141C Ins/Del						
Alcohol-dependent	103	58,3 (60)	41,8 (43)	0	79,1	20,9
Control	111	41,8 (43)	42,3 (47)	0	78,8	21,2
P value (<0.05)	X^2=0,19 P=0,90					
40 bp VNTR DAT1						
	N	**10/10 (N)**	**Others genotypes**		**Allele 10**	**Others alleles**
Alcohol-dependent	103	26,2 (27)	73,8 (76)		26,2	73,8
Control	111	31,5 (35)	68,5 (76)		31,5	68,5
P value (<0.05)	X^2=2,79 P=0,25					

Table 3: Gene and genotypic frequencies of the TaqIA, TaqIB and -141C Ins/Del polymorphisms in the alcohol dependence group with AUDIT scores of 14-19.

Gen and Group	N	Genotype Frequency % (n)			Gene frequency%	
		1/1	1/2	2/2	1	2
TAQIA						
Alcohol-dependent (AUDIT 14-19)	32	9,4 (3)	43,8 (14)	46,8 (15)	31,3	68,8
Control	32	9,4 (3)	40,6 (13)	50,0 (16)	29,7	70,3
P Value(<0,05)	X^2=0 P=1					
TAQIB						
Alcohol-dependent (AUDIT 14-19)	32	6,3 (2)	34,9 (36)	59,4 (19)	23,4	76,6
Control	32	21,9 (7)	26,1 (29)	53,1 (17)	34,4	65,6
P Value(<0,05)	X^2=2,870 P=0,238					
141C Ins/Del						
Alcohol-dependent(AUDIT 14-19)	32	65,6 (21)	34,4 (11)	0	82,8	17,2
Control	32	18,6 (6)	81,2 (26)	0	59,4	40,6
P Value(<0,05)	**X^2=10,32 P=0,005**					

polymorphisms. It is important to note that, of the 214 individuals genotyped for the DRD2 gene (polymorphism -141C Ins/Del), none of them presented the Del/Del genotype. Regarding the ORs for polymorphisms reported in the literature, the risk was not significant (Table 5). In the alcohol-dependent population, the TaqIA and TaqIB polymorphisms were in Hardy-Weinberg equilibrium. The -141C Ins/Del polymorphism of DRD2 was in disequilibrium (P=0.0029), such as the 40bp VNTR (P=0.0001) for a deficit among heterozygotes. Therefore any conclusion regarding these molecular markers must be interpreted with caution. Linkage disequilibrium for the -141C Ins/Del and TaqIB polymorphisms was calculated for located on the same DRD2 gene (promoter region and exon 1, respectively), and the results did not show a significant difference (P = 0.69), allowing for the inference that the alleles of these were two independently segregating polymorphisms. Similarly, TaqIA analysis for the polymorphism located in the ANKK1 gene and the -141C Ins/Del polymorphism of the DRD2 gene was performed (these genes are adjacent), and the results showed no significant difference (P = 0.94), indicating independent allele segregation. The gene and genotypic frequencies of individuals with alcohol dependence classified by an AUDIT score of 14-19 can be observed in Table 3. No significant differences in the TaqIA, 40bp VNTR or TaqIB polymorphisms were found. For the -141C Ins/Del polymorphism, significant differences (P=0.005) were found, with the risk Ins allele frequency calculated at 83% for the group with alcohol dependence, compared to 60% in the group with occasional consumption. With P = 0.01, an OR of 3.30 (1.45 to 7.47) was obtained. A score of 14 to 19 on the AUDIT indicates harmful drinking, so one could assume that carrying the Ins allele and having a score higher than 13 were associated with alcohol dependence. The OR´s for all polymorphisms are shown in Table 5. The distributions of the allele and genotype frequencies of the alcohol-dependent women are shown in Table 4. The results showed significant differences (P=0.05) for the TaqIB polymorphism, with alcohol-dependent women showing a frequency of 36% for B1 the risk allele, compared to 19% in the group of women with occasional consumption. The OR was 2.42 (1.04 to 5.66) for the B1 allele with P = 0.06, which is close to the limit of significance that could be deduced from what could be a risk allele in women with alcohol dependence in the population studied. Moreover, among men, the two groups did not show significant differences between gene and genotype frequencies among the four polymorphisms. When comparing the groups with regard to alcohol dependence and family history, no significant differences were found for polymorphisms.

The TaqIA allelic polymorphism frequency found for A1 (37.4%) was less than that observed in the Indian American population (58.3%) and in Mexican-Americans (45%), equivalent to that for Spanish (32.7%) and Japanese (39.5%) populations and higher than that observed in French (25.4%), other European (27.5%) and Brazilian (27.0%) populations. Regarding the B1 allele of the TaqIB polymorphism, the estimated frequency of 31.1% was still higher than that observed in the Indian population (25%) but less than estimated in the Mexican-American (40.5%) and Taiwanese (62%) populations [14,21,30,31,35,36,35,36]. The TaqIA polymorphism has been the most evaluated genetic polymorphism in alcohol dependency. Blum et al. in 1990 [27] reported the presence of the A1 allele of the DRD2 receptor gene in 77% of alcohol-dependent subjects, demonstrating an association of the A1 allele with alcoholism. Association studies of polymorphisms and alcohol consumption have remained ambiguous due to conflicting results. Thus, reconciling these ambiguous results would be the next step in determining whether associations with TaqIA are due to its own functionality or linkage disequilibrium with another gene [37]. In the present study, there were no significant differences in the polymorphism TaqIA to compare people with problematic alcohol and the no problematic use of the population, when stratification was made. The polymorphism identified as the -141C Ins /Del allele had a frequency of 79.1%, very close to that observed in the Indian population (77%) but lower than that estimated in the Japanese population (86%) and in Germany (91.1% estimated). It is important to note that, of the 214 individuals genotyped, none of them presented the Del/Del genotype. The frequency obtained for the 10R polymorphism, identified as corresponding to 10 repeats of 40 bp of the VNTR allele of the gene, was 26.2% in the group of alcohol-dependent individuals, which was observed to be much lower than in other populations: 63% in a Japanese

Table 4: Comparison of alcohol-dependent groups of women and women with casual consumers.

Gene and group	N	Genotype frequency % (n)			Gene frequency%	
		1/1	1/2	2/2	1	2
TAQIA						
Alcohol-dependent (Women)	29	13,79 (4)	41,38 (12)	44,83 (13)	34,48	65,52
Control	29	3,45 (1)	34,48 (10)	62,07 (18)	20,69	79,31
P value (<0.05)	X^2=3,847 P=0,146					
TAQIB						
Alcohol-dependent (Women)	29	20,69 (6)	31,03 (9)	48,28 (14)	36,25	63,79
Control	29	6,90 (2)	24,14 (7)	68.96 (38)	18,96	81,04
P value (<0.05)	**X^2=5,638 P=0,05**					
-141C Ins/Del						
Alcohol-dependent (Women)	29	62,07 (18)	37,93 (11)	0.00 (0)	81,03	18,97
Control	29	62,07 (18)	37,93 (11)	0.00 (0)	81,03	18,97
P value (<0.05)	X^2=0,00 P=1,00					

Table 5: Calculation of odds ratios for the risk alleles of the 4 polymorphisms in the alcohol-dependent group (all patients), in the alcohol-dependent group with AUDIT scores of 14-19 and alcohol-dependent women.

Group	Gene-allele	Odds ratio (95% CI)	Significance
All patients alcohol-dependent	ANKK1-TAQA1	1,41 (0,94-2,11)	p=0,10
	DRD2-TAQB1	1,09 (0,72-1,65)	p=0,76
	DRD2 -141C Ins	1,01 (0,63-1,62)	p=0,96
	-10R	0,77 (0,43-1,40)	p=0,25
Alcohol-dependent (AUDIT 14-19)	ANKK1-TAQA1	1,07 (0,51-2,28)	p=1,00
	DRD2-TAQB1	0,58 (0,26-1,26)	p=0,24
	DRD2 -141C Ins	**3,30 (1,45-7,47)**	**p=0,01**
	-10R	1,51 (0,55-4,21)	p=0,60
Alcohol-dependent women	ANKK1-TAQA1	2,02 (0,87-4,64)	p=0,14
	DRD2-TAQB1	**2,42 (1,04-5,66)**	**p=0,06**
	DRD2 -141C Ins	1,00 (0,39-2,53)	p=0,82
	-10R	1,11 (0,35-3,53)	p=0,90

population, 92% in Taiwanese subjects, 44% in Italians, 51% in the French and 35% in Brazilians (14; 26).The gene might be considered a candidate gene for vulnerability to substance abuse. Tiihonen et al [38] suggested that, which plays an important role in uptake, was altered because of the carrier density required to take CT scans for single photon emission (SPECT) in non-violent alcohol-dependent individuals. Although VNTR resides at the 3' UTR end and therefore does not affect the amino acid sequence of the protein, regulatory factors, such as the stability of the mRNA, nuclear transport and protein synthesis, are potentially affected by these variations [39] The mechanism by which the availability of DAT transporter is regulated in the brain is not yet known exactly. Neuro imaging studies have shown that levels of DAT might be affected by the chronic use of alcohol, and DAT levels were significantly lower in the striata of alcohol-dependent humans than in controls, returning to control levels after a period of abstinence [38]. To date, several association studies have been performed to evaluate the contribution of the 40-bp VNTR in the development of alcohol dependence, with conflicting results. By stratifying the alcohol-dependent population by AUDIT score, significant differences were obtained for the Ins -141C allele of the DRD2 gene in individuals with score of 14 to 19 (Table 3). Classification by AUDIT in 3 domains was performed: risk consumption of alcohol, dependence symptoms and harmful alcohol consumption. Scores between 14 and 19 indicate an individual engaging in harmful drinking who would benefit from brief therapy and a continuous approach (PRADICAN, 2012). The -141C Ins/Del polymorphism being located in the DRD2 gene promoter plays an important role in the expression of the D2 receptor. In vitro analyses have suggested that this polymorphism alters the transcriptional activity of the gene and thus regulates the expression [13]. An imaging study conducted in healthy volunteers showed an increase in the density of striatal receptor allele in individuals with -141C [40]. A genetic association study performed by Samochowiec et al. [41] suggested a possible role in the development of alcohol addiction for the German -141C genotype. Similarly the results obtained were in accordance with those observed in a Korean population in which alcohol-dependent individuals classified with the Lesch typology were compared with healthy individuals and with an Indian population [42]. Therefore, a more meaningful representation of the -141C Ins allele in alcoholic subjects with representative AUDIT scores in the study population could be correlated with a possible decrease in the density of D2 receptors in patients with alcohol dependence, in turn stimulating the desire to drink more and in greater amounts to be rewarded, thus promoting alcohol dependence. When comparing gender, significant differences were found for the IB allele of the TaqIB polymorphism in the group of women with alcohol dependence and the group of women with occasional alcohol consumption. In the calculation of OR (OR = 2.42 (1.04 to 5.66)), a value of P = 0.06, which is close to the limit of significance, could suggest that B1 is a risk allele for alcohol dependence among women in the study population. The TaqIB polymorphism, located within the coding region of the DRD2 gene, plays an important role in the function of this gene. The study reported by Konishi et al. in a Mexican-American population reported conflicting results regarding the association of this polymorphism with alcohol dependence. The study by O'Hara et al. [11] established significant differences between blacks and Caucasians, concluding that a lower frequency of the B1 polymorphism in the black population, compared to Caucasians [12]. Thus far, there have no reports of an association between this polymorphism and alcohol dependence in the female population as such. The only report concerning the sex of the individual was published by Lu et al in 1996, and these authors assessed the associations of the polymorphism in a population of single men from three different regions of Taiwan, finding significant differences. A study by Méndez in 2009 [43] of the Colombian population (similar to that used in this study) typified polymorphisms related to the metabolism of alcohol, finding significant differences for the ADH2 polymorphism when comparing a group of women with problematic alcohol consumption and the respective control group. Importantly, it has not been established that this polymorphism (ADH2) is related a polymorphism of the dopaminergic system. In addition, a significant difference was found between the group with alcohol dependence and the occasional consumption group regarding family history, allowing us to assume a role for family influence in

the problem of alcohol addiction. A relationship between the consumption of alcohol and other addictive substances was also found. Sixty-six percent of individuals with alcohol dependence and 18.0% of individuals with occasional alcohol consumption responded affirmatively, showing very significant differences between the two groups (P = 0.001). The most used substances by the group with alcohol dependence were marijuana, cocaine and crack cocaine. Alcohol dependence often coexists with other addictions, including substance abuse and nicotine dependence, and with such psychiatric illnesses as depression, anxiety, antisocial personality disorder, and conduct disorder, among others. Such comorbidity between disorders could indicate the existence of etiological factors that are shared. In connection with alcohol dependence, different studies conducted worldwide have differed in the relationships of polymorphisms of the dopaminergic system and their roles in the development of addiction. Physiologically, alcohol consumption increases the synaptic dopamine level, which reinforces self-administration. In the ventral striatum, especially in the NAc, drugs of abuse stimulate dopamine release, reinforcing exaggerated consumption. Decreases in the numbers of dopamine receptors in the pre- and postsynaptic neurons have been associated with the desire to drink greater amounts of alcohol and an increase in the processing of alcohol related stimuli in the prefrontal cortex [44]. The above observations must be tested in subsequent studies, in which the number of subjects studied is greater and the diagnostic criteria for alcohol dependence include a classification by type of alcoholism. Most of the studies described had a case-control design, in which all alcohol-dependent individuals are treated as a single group. Therefore, the identification of subgroups and the clinical heterogeneity of alcohol dependence, if considered, could represent reality more authentically and could increase the ability to find associations [45,46]. Furthermore, the definition of the subgroups or endo phenotypes closely associated with a particular genetic factor or a generally alcohol-dependent phenotype could result in more robust and reproducible results if the subgroups are sizable.

CONCLUSION

In the present study no association was found between polymorphism of DRD2 gene Taq1A with alcoholism in this Colombian population. The possible relationships of polymorphisms that we evaluated with alcohol dependence were obtained for the group with AUDIT scores between 14 and 19 were studied, and the frequency of the -141C Ins allele was significant, compared with the control group. The present study suggested that the Ins - 141C allele was critical to the harmful phase of dependent alcohol consumption. Similarly, a significant difference in the IB allele of TaqIB polymorphism of the DRD2 gene was found in the group of women with alcohol dependence, but it is recommended to increase the sample size to confirm this conclusion.

The answers reported by the participants to the study questionnaire clarified that alcohol-dependent individuals are also more likely to use other psychoactive substances. Similarly, there were a greater number of individuals who claimed to have family with problematic alcohol consumption in the group with problem drinking than in the control group, suggesting the influence of genetic disease.

Of the 214 individuals genotyped for the DRD2 gene (polymorphism -141C Ins/Del), none of them presented the Del /Del genotype.

ACKNOWLEDGEMENTS

- To all foundations for opening its doors (Fundación el Pacto, Fundación La Luz, Grupo de Alcohólicos Anónimos and Asociación Comunidad Terapéutica, COTECOL).
- To all of those who gave us 15 minutes of your time to complete the consent form and questionnaire and to participate in the study
- Thanks to the Faculty of Medicine at the National University of Colombia for project financing

REFERENCES

1. Herrán OF, Ardila MF, Barba DM. [Test for assessing levels of alcohol consumption in Bucaramanga, Colombia: design and validation]. Biomedica. 2008; 28: 25-37.
2. Keriel-Gascou M, Figon S, Letrilliart L, Chaneliére M, Colin C. [Classifications and definitions of adverse events in primary care: a systematic review]. Presse Med. 2011; 40: e499-505.
3. PRADICAN: Anti-Illegal Drugs Programme of the Andean Community. Andino II epidemiological study on Drug Use in University City. Regional Report. 2012.
4. Rehm J, Monteiro M. Alcohol consumption and burden of disease in the Americas: implications for alcohol policy. Rev Panam Salud Publica. 2005; 18: 241-248.
5. Monteiro MG. Alcohol and Public Health in the Americas: a case for action. 2007; 1-49.
6. Heinz A, Goldman D, Gallinat J, Schumann G, Puls I. Pharmacogenetic insights to monoaminergic dysfunction in alcohol dependence. Psychopharmacology (Berl). 2004; 174: 561-570.
7. Cardinal RN, Parkinson JA, Hall J, Everitt BJ. Emotion and motivation: the role of the amygdala, ventral striatum, and prefrontal cortex. Neurosci Biobehav Rev. 2002; 26: 321-352.
8. Heinz A. Dopaminergic dysfunction in alcoholism and schizophrenia--psychopathological and behavioral correlates. Eur Psychiatry. 2002; 17: 9-16.
9. Schuckit MA. Vulnerability factors for alcoholism. Neuropsychophamacology, Lippincott Williams and Wilkins. Philadelphia. 2002.
10. Hauge XY, Grandy DK, Eubanks JH, Evans GA, Civelli O, Litt M. Detection and characterization of additional DNA polymorphisms in the dopamine D2 receptor gene. Genomics. 1991; 10: 527-530.
11. O'Hara BF, Smith SS, Bird G, Persico AM, Suarez BK, Cutting GR, et al. Dopamine D2 receptor RFLPs, haplotypes and their association with substance use in black and Caucasian research volunteers. Hum Hered. 1993; 43: 209-218.
12. Smith S, O'Hara B, Persico A, Gorelick A, B Newlin, Vlahov D, et al. Genetic vulnerability to drug abuse. The D2 dopamine receptor Taq I B1 restriction fragment length Polymorphism Appears faq frequently in polysubstance abusers more. Arch Gen Psychiatry 1992; 49: 723-727.
13. Arinami T, Gao M, Hamaguchi H, Toru M. A functional polymorphism

in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Hum Mol Genet. 1997; 6: 577-582.

14. Chen WJ, Chen CH, Huang J, Hsu YP, Seow SV, Chen CC, et al. Genetic polymorphisms of the promoter region of dopamine D2 receptor and dopamine transporter genes and alcoholism among four aboriginal groups and Han Chinese in Taiwan. Psychiatr Genet. 2001; 11: 187-195.

15. Parsian A, Cloninger CR, Zhang ZH. Functional variant in the DRD2 receptor promoter region and subtypes of alcoholism. Am J Med Genet. 2000; 96: 407-411.

16. Sander T, LadehoffM, Samochowiec J, Finckh U, Rommelspacher H, Schmidt LG. Lack of an allelic association of the polymorphisms Between dopamine D2 receptor gene and alcohol dependence in the German population. Clin Exp Alcohol Res. 1999; 23: 578-581.

17. Neville MJ, Johnstone EC, Walton RT. Identification and characterization of ANKK1: a novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. Hum Mutat. 2004; 23: 540-545.

18. Laakso A, Pohjalainen T, Bergman J, Kajander J, Haaparente M, Solin O, Syvalahti E, et al. The A1 allele of the human D2 dopamine receptor gene is Associated with Increased activity of L-amino acid strialdescarboxylase in healthy subjects. Pharmacogenet Genomics 2005; 15: 387-391.

19. Thompson J, Thomas N, Singleton A, Piggott M, Lloyd S, Perry EK, et al. D2 dopamine receptor gene (DRD2) Taq1 A polymorphism: reduced dopamine D2 receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics. 1997; 7: 479-484.

20. Pinto E, Reggers J, Gorwood P, Boni C, Scantamburlo G, Pitchot W, et al. The TaqI A DRD2 polymorphism in type II alcohol dependence: a marker of age at onset or of a familial disease? Alcohol. 2009; 43: 271-275.

21. Bau C, Almeida S, Hutzs M. TheTaqI A1 Allele of the Dopamine D2 Receptor Gene and Alcoholism in Brazil: Association and Interaction with Stress and Harm Avoidance on Severity Prediction. Am J Med Genet (Neuropsychiatric Genetics) 2000; 96: 302-306.

22. Giros B, Caron MG. Molecular characterization of the dopamine transporter. Trends Pharmacol Sci. 1993; 14: 43-49.

23. Reith ME, Xu C, Chen NH. Pharmacology and regulation of the neuronal dopamine transporter. Eur J Pharmacol. 1997; 324: 1-10.

24. Elsworth JD, Roth RH. Dopamine synthesis, uptake, metabolism, and receptors: relevance to gene therapy of Parkinson's disease. Exp Neurol. 1997; 144: 4-9.

25. Drgon T1, Lin Z, Wang GJ, Fowler J, Pablo J, Mash DC, Volkow N. Common human 5' dopamine transporter (SLC6A3) haplotypes yield varying expression levels in vivo. Cell Mol Neurobiol. 2006; 26: 875-889.

26. Heinz A, Goldman D, Jones DW, Palmour R, Hommer D, Gorey JG, et al. Genotype influences in vivo dopamine transporter availability in human striatum. Neuropsychopharmacology 2000; 22: 133-139.

27. Blum K, Noble EP, Sheridan PJ, Montgomery A, Ritchie T, Jagadeeswaran P, et al. Allelic association of human dopamine D2 receptor gene in alcoholism. JAMA. 1990; 263: 2055-2060.

28. Comings DE, Comings BG, Muhleman D, Dietz G, Shahbahrami B, Tast D, et al. The dopamine D2 receptor locus as a modifying gene in neuropsychiatric disorders. JAMA. 1991; 266: 1793-1800.

29. Berggren U, Fahlke C, Aronsson E, Karanti A, Eriksson M, Blennow K, et al. The taqI DRD2 A1 allele is associated with alcohol-dependence although its effect size is small. Alcohol Alcohol. 2006; 41: 479-485.

30. Goldman D, Brown L, Albaugh B, Robin R, Goodson S, Trunzo M, et al. DRD2 dopamine receptor genotype, linkage disequilibrium, and alcoholism in American Indians and other Populations. ClinExp Alcohol Res 1993; 17: 199-204.

31. Konishi T, Calvillo M, Leng AS, Lin KM, Wan YJ. Polymorphisms of the dopamine D2 receptor, serotonin transporter, and GABA(A) receptor beta(3) subunit genes and alcoholism in Mexican-Americans. Alcohol. 2004; 32: 45-52.

32. Babor TF, Higgins-Biddle JC, Saunders JB, Monteiro MG. AUDI: Identifying questionnaire use disorders Alcohol. Geneva : World Health Organization . Department of Mental Health and Substance Dependence ; 2001 .

33. Whatman, CloneSaver Card Instructions (with FTA®Technology) Cat No. WB12 0028.FTA® Whatman® Protocols.Collect, Transport, Archive and Access Nucleic Acids. Whatman; 2002.

34. Raymond M, Rousset F. GENEPOP Version 1.2: population genetics software for exact tests and ecumenicism.J. Hered. 1995; 86: 248-249.

35. Rubio G, Jimenez M, Ponce G, Hoenicke J, Ampuen L, Rodriguez R, et al. Review of clinical significance TaqI polymorphism in alcohol dependence. Psychiatric Bama Magazine Fac Med. 2004; 3: 141-150.

36. Ishiguro H, Saito T, Akazawa S, Mitushio H, Tada K, Enomoto M, et al. Association Between drinking-related antisocial behavior and a polymorphism in the serotonin transporter gene in a Japanese population. Alcohol Clin: Exp Res. 1999; 23: 1281-1284.

37. Lucht M, Barnow S, Schroeder W, Grabe HJ, Rosskopf D, Brummer C, et al. Alcohol consumption is associated with an interaction between DRD2 exon 8 A/A genotype and self-directedness in males. Neuropsychobiology. 2007; 56: 24-31.

38. Tiihonen J, Kuikka J, Bergström K, Hakola P, Karhu J, Ryynänen OP, et al. Altered striatal dopamine re-uptake site densities in habitually violent and non-violent alcoholics. Nat Med. 1995; 1: 654-657.

39. Fuke S, Sasagawa N, Ishiura S. Identification and characterization of the Hesr1/Hey1 as a candidate trans-acting factor on gene expression through the 3' non-coding polymorphic region of the human dopamine transporter (DAT1) gene. J Biochem. 2005; 137: 205-216.

40. Jönsson EG, Nöthen MM, Grünhage F, Farde L, Nakashima Y, Propping P, et al. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Mol Psychiatry 1999; 4: 290-296.

41. Samochowiec J, Ladehoff M, Pelz J, Smolka M, Schmidt LG, Rommelspacher H,et al. Predominant influence of the 3'-region of dopamine D2 receptor gene (DRD2) on the clinical phenotype in German alcoholics. Pharmacogenetics. 2000; 10: 471-475.

42. Suraj Singh H, Ghosh PK, Saraswathy KN. DRD2 and ANKK1 gene polymorphisms and alcohol dependence: a case-control study among a Mendelian population of East Asian ancestry. Alcohol Alcohol. 2013; 48: 409-414.

43. Méndez C. Characterization of polymorphisms of cytochrome P4502E1 and Alcohol dehydrogenase genes in a population of Bogotá and evaluation of their possible relationship to the problematic use of alcohol. National University of Colombia. 2009.

44. Kienast T, Heinz A. Dopamine and the diseased brain. CNS Neurol Disord Drug Targets. 2006; 5: 109-131.

45. Cruz C, Camarena B, Mejía JM, Páez F, Eroza V, Ramón De La Fuente J, et al. The dopamine D2 receptor gene TaqI A1 polymorphism and alcoholism in a Mexican population. Arch Med Res. 1995; 26: 421-426.

46. Laine TP, Ahonen A, Torniainen P, Heikkla J, Pyhtinen J, Rasanen P, et al. Dopamine transporters Increase in human brain after alcohol withdrawal. Molecular Psychiatry. 1999; 4: 189- 191.

Cite this article

Castro Matiz TX, Aristizabal Gutierrez FA, Buitrago MR (2015) Determination of Genetic Polymorphism Taqia (Ankk1) Taqib (Drd2), -141c Ins/Del (Drd2) And 40 Bp Vntr (Slc6a3) in the Colombian Population and Evaluation of their Associations with Alcoholism. J Subst Abuse Alcohol 3(4): 1039.