The Genetic Analysis of High Risk Athletes for the Presence of the DRD4 7 Variable Tandem Repeat

Dorian Yablin
Dr. Nancy L. Elwess **(Faculty)**

Department of Biological Sciences
State University of New York at Plattsburgh

ABSTRACT

This study examined the DRD4 tandem repeats within three self-described risk-taking groups ranging in expertise from students in the Expeditionary Studies program to internationally ranked Olympic Bobsledders. Prior studies done on novelty seeking have had conflicting results on the 7 variable tandem repeat of the Dopamine Receptor D4. The goal of this study was to find a statistically significant relationship between the 7+ repeat and its possible genetic influence on risk taking behavior. Results indicated that when the experimental group was compared to the control, the 7+ repeat did influence an individual's willingness to take risk. When compared separately, both the Olympic Bobsledders and Free Soloing Rock Climber groups had statistically significant results. The Expeditionary Studies group by itself was not statistically significant in the amount of 7+ repeats present. Professional certifications in the experimental groups were not taken into account for this study.

Keywords:
DRD4, dopamine, risk takers, thrill seekers, tandem repeats

INTRODUCTION

Dopamine receptors (DR) consist of G-protein-coupled receptors. The function of these receptors is to bind to extracellular dopamine producing a reaction within different parts of the brain (Keeler, Pretsell, & Robbins, 2014). The dopamine receptor D4 (DRD4) expresses a polymorphism in exon III; the variants in this exon are caused by allelic variations on the 11th chromosome (Figure 1). Changing the number of possible repeats on exon III of the D4 receptor alters the length, structure, and the efficiency of the receptor (Sander et al., 1997). DRD4 has ten known repeats between 2 and 11 within this exon. The most common repeats being 2, 4 and 7 (Haradon & Galdzicki, 2015).

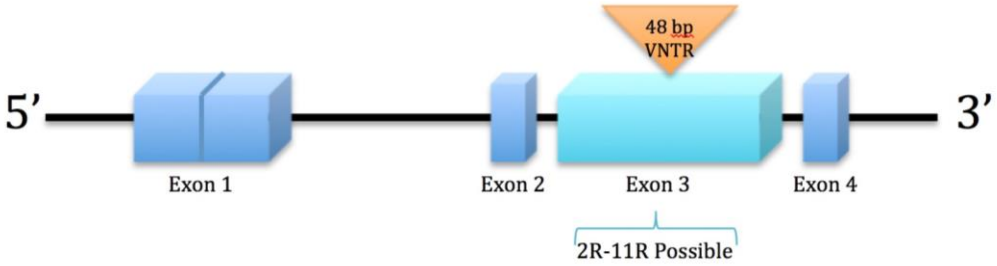

Figure 1: Dopamine receptor D4 gene. The 48 bp tandem repeat is located within exon 3. Image generated by Dorian Yablin.

There are two dopamine receptor families, D1 and D2. The D1 family's includes DR 1 and 5. Their primary function is to send excitatory signals, increasing the chance of a given D1 medium spiny neuron firing. The D2 family includes DR 2, 3 and 4 and behaves in an inhibitory fashion, opposing the D1 excitatory signal. The D2 family is coupled to second messenger systems through inhibitory G-proteins and is structurally more complex than the D1 family. Due to the increased complexity of the D2 family, there are different types of each D2 receptor, unlike the D1 receptors (Meador-Woodruff, 1998). When the brain receives a stimulatory signal, dopamine plays an important role in how the brain tells the body to react. This learned reaction for a certain stimulus is known as reinforcement learning (Montague, Hyman, & Cohen, 2004). When award succeeds an action in the brain, it is able to recognize the pleasurable feeling, and is reinforced in future situations. Reinforcement learning is the brain's way of trying to repeat an activity due to the favorable release of dopamine that is released (Keeler et al., 2014).

Studies conducted in the last twenty years have associated the 7 repeat variant of the DRD4 (DRD4-7R) with individuals expressing high levels of novelty seeking (Sander et al., 1997) (Benjamin et al., 1996). Novelty seeking is viewed as a non-dysfunctional behavior, and is positively correlated with "volumes of frontal and posterior cingulate cortices" (Laricchiuta et al., 2014). Individuals who scored higher than average on the Tridimensional Personality Questionnaire (TPQ) were characterized as impulsive, exploratory, and excitable. Individuals in a similar study who scored higher than average in a Novelty Seeking test expressed the DRD4-7R variant (Ebstein et al., 1996). The DRD4-7R variant has been associated with behavioral impulsivity, risk taking, and novelty seeking in both humans and other animals (Coyne et al., 2015). Researchers have also been able to associate the longer 6-8 repeats with individuals who have higher novelty seeking and risk taking abilities than those with the shorter 2-5 repeat alleles (Cloninger, Svrakic, & Przybeck, 1993).

The focus of this research was to find a genetic connection in regard to the number of variable tandem repeats in the DRD4 gene and individuals who participate in high-risk activities as compared to a control group. The first experimental group (n=26), was a group of collegiate students in the Expeditionary Studies major. In this major, students spend four years learning how to be self-sufficient in the backcountry. They spend an extended amount of time outside doing high-risk sports such as whitewater kayaking, rock climbing, ice climbing, and backcountry skiing. These students must also complete a capstone expedition, where they execute a 10 day self-sustaining trip based on minimal to no assistance, and rely solely on the skills they have learned during their academic career. The second experimental group (n=18) was provided by a group of international bobsledders competing in an international competition in Lake Placid, NY (Figure 2). This was considered a good experimental group due to the amount of risk associated with them reaching speeds of 85mph on the track during their competitions. The third experimental group (n=9) were free rock climbers, also known as Free Soloing. This is a form of rock climbing where they do not rely on any equipment or ropes. This group was taking part in a climbing event held in Lake Placid, NY. The fourth group (n=38) was the control. This group consisted of students from a Bio 101 General Biology class that were not in the Expeditionary Studies major. Every individual has two copies of this gene, one from their mother and one from their father. Genotypic results for each individual were based on the presence of the 7 repeat, or higher allele (7^+) or absence of a 7 repeat (7^-). This study was designed to examine the extent in which the tandem repeats appeared each group and to determine if they played a role in the novelty seeking/thrill seeking activities of each group. The hypothesis was that the three experimental groups (expeditionary study majors, international bobsledder, and free rock climbers) would display a statistically higher frequency of 7^+ repeats than the control.

Figure 2. One of the test groups were international bobsledders competing at an international bobsled event in Lake Placid, NY. Image taken by Kindsley Dodson.

METHODS

The Human Subjects Research Review Committee at the State University of New York at Plattsburgh approved all procedures used for this project. All participants signed consent forms with the option to withdraw from the study at any time.

Cheek cells were collected from 91 participants using 0.9% saline solution (Table 1). Their DNA was then isolated from the solution using the procedures as outlined in Genetic Origins (Cold Spring

Harbor Laboratory, 2016). The DRD4 polymorphic genotypes were verified through polymerase chain reaction (PCR) as described in Dreber et al. (2009). The PCR primers used were: 5'- GCG ACT ACGTGG TCT ACT CG -3' and 5'- AGG ACC CTC ATG GCC TTG -3'. Previous research results discussed problems with consistent genotyping of the tandem repeat region within the DRD4 gene (Eisenberg et al., 2008; Eisenberg et al., 2007; and Hamarman et al., 2004). Due to multiple bands showing up when only two, one for each allele should appear. Dreber et. al (2009) was helpful in solving this problem by discussing their use of dITP to prevent unnecessary bands from appearing in the gels.

Table 1. Number of Participants in each group tested

Control	Expeditionary majors	International bobsledders	Free rock climbers
38	26	18	9

The PCR products were visualized using ultraviolet fluorescence following migration of samples within 1.2% agarose gels containing ethidium bromide, following the protocol as explained in Lichter et al. (1993).

STATISTICAL ANALYSIS

Analysis was done using cross tabulation through SPSS. Participants were classified according to the presence of (7^+) or the absence (7^-) of the repeats founds within exon III of the DRD4 gene (Figure 3, Table 2).

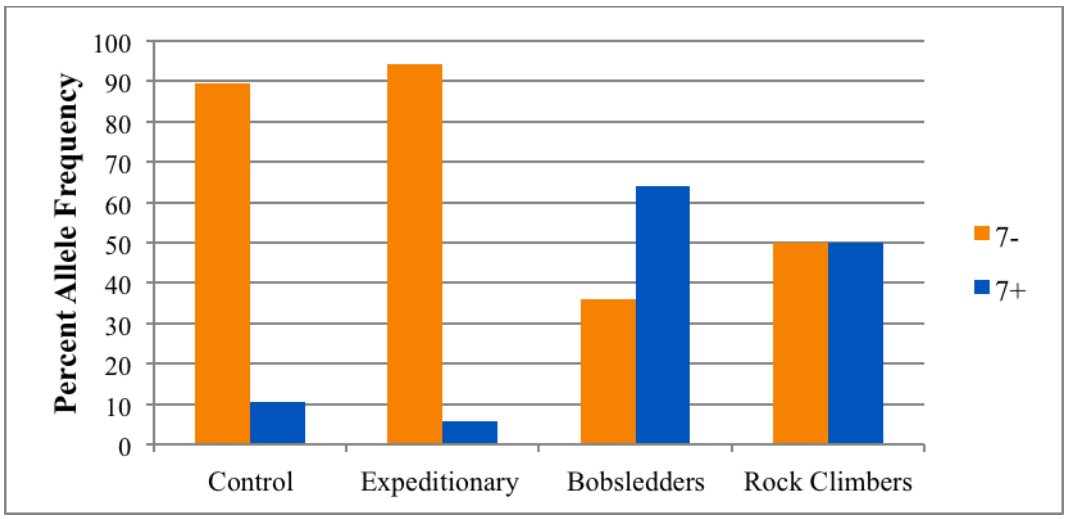

Figure 3. This figure represents the allele frequency in each group of participants.

Table 2. Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	55.698[a]	3	.000
Likelihood Ratio	52.880	3	.000
Linear-by-Linear Association	44.409	1	.000
N of Valid Cases	182		

RESULTS

A total of 91 participants were examined in all four groups combined. 7 of the 38 individuals within the control group had at least one copy of the 7^+ variant; representing 18.42% of the total control sample. In the expeditionary majors only 2 of the 26 individuals had at least one copy of the 7^+ variant, representing 7.69% of the total group. The two other experimental groups displayed genetically distinct 7^+ results. 6 of the 9 rock climbers had at least one copy of the 7^+ variant (66.66%), while 15 of the 18 international bobsledders had at least one copy of the 7^+ variant (83.33%). When the overall allele frequencies were calculated for each group, it was unexpected to find that the expeditionary majors had the lowest 7^+ allele frequency at only 5.8%. However, the international bobsledding test group had a 63.9% allele frequency of the 7^+ allele frequency (Figure 3). Despite the low presence of the 7^+ allele frequency within the expeditionary majors, overall when the three test experimental groups were compared to the control group, there was a significantly higher amount of 7^+ repeats (Table 2, Figure 4).

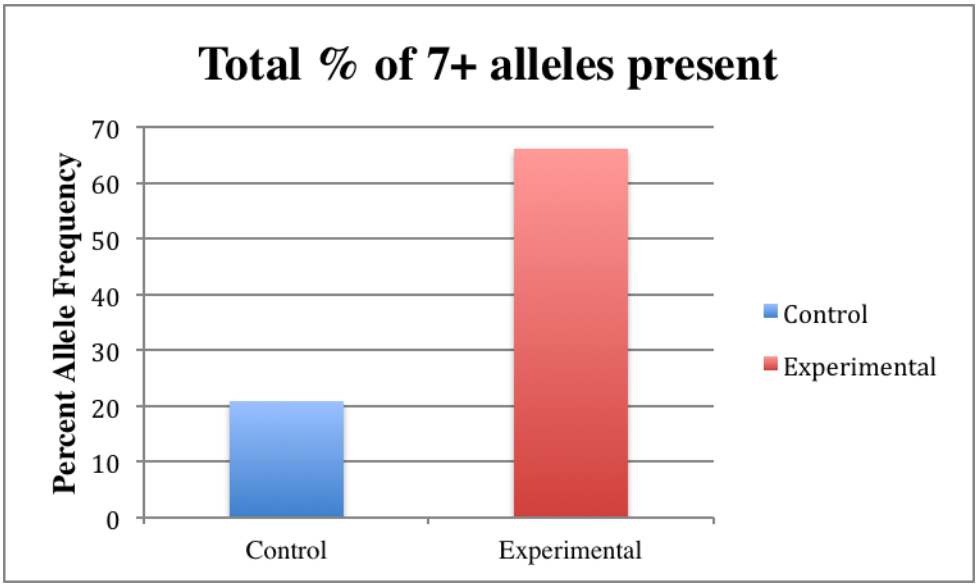

Figure 4: The comparison of the control group to the three experimental groups combined.

DISCUSSION

The three experimental groups were chosen specifically for this study based on the amount of risk taking that is associated with each group, and the personalities of the individuals drawing them to participate in higher risk activities. When analyzing the results, it was unexpected to see such low 7^+ repeat results for the Expeditionary Studies group. In the survey responses taken by all four groups, 65% of the expeditionary studies group said they would be very likely to take risk when participating in higher risk sports such as rock climbing, or white water kayaking (Table 3). After isolating and analyzing the participant's saliva samples, only 7.69% of the participants contained a single or double allele of the 7^+ repeat variant (Table 2, Figure 3).

Table 3: Survey Results of Expeditionary Studies students and the Control

	Survey Response: likelihood of taking High Risk	Genetic Results
Expeditionary Studies Students	65%	7.7%
Control Population	14%	18.4%
Olympic Bobsledders	100%	83.3%
Free soloing rock climbers	100%	66.6%

As the level of risk and experience increased to free soloing rock climbers and Olympic level bobsledders there was a greater amount of individuals expressing one or both 7^+ alleles. Overall there was a significantly higher amount of 7^+ repeats present when the three experimental groups were combined (table 2). There was no statistically significant difference between the 7^+ repeat variant and the Expeditionary Studies group when compared to the control group separately (figure 3). Similar results were found in Sander's research on novelty seeking, conducted in 1997 (Sander et al., 1997). There are many different possibilities as to why the research yielded an insignificant difference between the Expeditionary Studies group, and the control. What was not taken into consideration during the study was the level of expertise the Expeditionary Studies group had. In the outdoor industry there are various types of certifications awarded to individuals who prove their ability to do certain high-risk sports under different circumstances. For example the American Canoe Association certifies white water kayakers on a scale of level 1 to level 5. Level one certified individuals are expected to perform the basic movements reasonably straightforward, in what would be considered safe conditions. Level 5 certified individuals are expected to know how to control their kayak, read the movements of the river, as well as watch over a group of kayakers in class II-IV rapids ("ACA Instructor Criteria," 2014). If this study were repeated, the survey would be more extensive, and would ask individuals about any certifications they have pertaining to these activities. It would also ask about participant's level of expertise in the sport, not just their willingness to take risk.

Both the international bobsledders and free soloing rock climbers are competing at the highest level of expertise in their sport. The Expeditionary Studies group is still learning the proper techniques to safely participate in the various high-risk sports.

ACKNOWLEDGMENTS

Thanks to Dr. Thomas Wolosz for his assistance with the statistical analysis.

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