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# Table of Contents

## Abstract

PURPOSE: The purpose of this study was to examine the genetic basis underlying voluntary exercise. Monoamine oxidase A (MAO-A) is an enzyme that acts on monoamine neurotransmitters, such as dopamine, to cause inactivation. There are several polymorphisms in the promoter region of the MAO-A gene and these variations change transcriptional activity and the amount of MAO-A produced, leading to alterations in available dopamine levels. Interestingly, polymorphisms in MAO-A have been associated recently with physical activity level. This study sought to determine whether there is an association between motivation to exercise, levels of voluntary physical activity and MAO-A gene polymorphisms. METHODS: Seventy-one participants (age 18-24 years, 13 males & 58 females) completed the Behavioral Regulation in Exercise Questionaire-2 (BREQ-2) to assess their motivation to exercise and the International Physical Activity Questionnaire (IPAQ) to assess their level of physical activity. DNA was collected and isolated from a cheek cell sample. The MAO-A genotype was identified using PCR with gene specific primers. MAO-A 3/3 and 4/4 genotype individuals were used for analysis. RESULTS: External motivation to exercise was significantly higher ($p < 0.01$) in the high transcription 4/4 genotype ($\bar{x}$=1.11 ± 0.8) compared to the low transcription 3/3 genotype ($\bar{x}$= 0.39 ± 0.6). Internal motivation to exercise was not different between genotypes. Body mass index and weekly MET minutes estimated by IPAQ were also comparable between genotypes. CONCLUSION: The results suggest a polymorphism in this monoamine pathway may play a role in increasing sensitivity to external factors that motivate individuals to exercise.

## Introduction

A healthy lifestyle includes participating in regular physical activity, which can reduce risk of heart disease, stroke, type II diabetes and depression. The Centers for Disease Control and Prevention (CDC) currently recommends that adults aged 18-64 reach a weekly aerobic and muscle-strengthening goal of two hours and 30 minutes of moderate aerobic activity, or one hour and 15 minutes of vigorous aerobic activity, along with muscle-strengthening activities 2 or more days a week. In the United States, only 20.8% of adults who are 18 or older meet the CDCs recommended guidelines for physical activity (CDC, 2014). There are a number of possible factors that explain low physical activity levels including, inadequate time, insufficient resources or negative affect toward exercise. Extrinsic motivators such as peer pressure can be used to initiate exercise programs, however, intrinsic motivators are needed to continue daily exercise protocols (Good, Li & Deater-Deckard, 2015). Intrinsic motivation is thought to involve dopamine transmission in the brain. Dopamine is a neurotransmitter that is part of the neurocircuitry that stimulates pleasure and regulates motivation to obtain pleasure (Goldfield et al., 2013). Humans are naturally pleasure seeking due to the central reward sensation that dopamine causes. Several studies have shown that genetic variations in the dopamine pathway affect the amount of voluntary exercise in mice. However, there are limited studies on these genetic variations and exercise levels in humans. Therefore, it is important to examine the potential interplay between dopaminergic genetic differences, exercise levels and specific motivational states in human participants as a means of understanding the biological basis for engagement in physical activity.

## Self-Determination Theory

Self-determination theory seeks to explain motivational behavior. According to this theory, humans are motivated to ensure that basic psychological needs are met in order to achieve optimal psychological health and well-being. The three needs are grouped into areas called competence, relatedness and autonomy (Deci & Ryan, 2000). Participation in sport and exercise can either help or harm one in reaching their psychological needs. For example, ability to perform a difficult physical task could either boost competence or greatly reduce perceived competence. Being part of an exercise group or sports team can lead to increased feelings of relatedness compared to working out alone. Finally, those who participate in physical activity for internal satisfaction may feel greater autonomy than those who engage in physical activity due to external pressures. Thus, motivation to exercise can be understood in the context of meeting these basic, underlying psychological needs (Teixeira, Carraça, Markland, Silva, & Ryan, 2012).

Participation in physical activity is driven by goal content or motives. Motives can be categorized based on the degree to which they satisfy basic psychological needs and are thought to serve as the loci of control for behavior (Ingledew & Markland, 2008). Motives may be intrinsic such as maintaining health, or to challenge oneself and are often closely related to accomplishing basic psychological needs. Conversely, an extrinsic goal, such as improving appearance, is not necessarily essential to well being. In this perspective, motives underlying health and physical activity may have both extrinsic and intrinsic qualities for a given activity (Teixeia et al. 2012). Self-determination theory claims that an individual is intrinsically motivated when an activity is performed due to satisfaction derived from the activity and externally motivated when the activity is performed for a reward or to avoid punishment. In contrast to external and internal motivation, amotivation occurs when an individual lacks any self-determination for physical activity. This may occur if they sense lack of control for the desired outcomes (Deci & Ryan, 2000).

## Physical Activity and Intrinsic Motivation

Behaviors that are intrinsically motivated are freely engaged in due to the interest of the individual, without external pressures and are maintained by the desire to satisfy the basic psychological needs. Autonomy is required in order for a behavior to be intrinsically motivated. However, autonomy can be undermined by extrinsic rewards and threats that decrease autonomy and shift the locus of causality to external. Competence is also essential to intrinsic motivation. Positive feedback has been linked to an increase in intrinsic motivation due to the increased sense of competence whereas negative feedback has been linked to a decrease in intrinsic motivation. Relatedness does not have quite the same influence as autonomy and competence on intrinsic motivation but it can increase intrinsic motivation. Relatedness is not as central because individuals often are intrinsically motivated to engage in behaviors in isolation (Deci & Ryan, 2000).

Physical activity that is driven by intrinsic motivation is typically engaged in for prolonged periods of time. Those who are driven by controlling motives or extrinsic motivation may be involved in the initiation of physical activity but are unlikely to maintain involvement in physical actively for extended periods of time (Friederichs, Bolman, Oenema, & Lechner, 2015).

**Physical activity and Extrinsic Motivation**

Within external motivation there is a continuum of behavioral regulations that can be divided into four categories. The continuum or categories are defined by the extent to which the behavior has been internalized or integrated into ones self (Ingledew & Markland, 2008). According to self-determination theory, internalization is a natural process that occurs when individuals transform socially sanctioned norms into personal values and self-regulations. The continuum or first category begins with external regulation, which is the standard example of extrinsic motivation and would present as doing something for reward or to avoid punishment. Introjected regulation is the next category and occurs when a behavior is partially internalized so that the individual now administers their own consequences, such as to boost their self-esteem or reduce guilt. Identified regulation is the third category and is defined as when an individual recognizes the value of the behavior. For example, someone may exercise because they value the health benefits of exercise. Integrated regulation is the fourth category, at the end of the continuum and is the most internalized form of extrinsic motivation. This occurs when a behavior is consistent with an individual's values. Once a behavior has become fully internalized it will have transformed from external regulation to self-regulation resulting in self-determined extrinsic motivation. As a behavior becomes more autonomous along the continuum it is associated with greater commitment and sustained engagement (Deci & Ryan, 2000).

### Dopamine's Involvement in Motivation

The limbic portion of the basal ganglia in the brain is responsible for an individual's response to motivational, emotional and contextual information that affects behavior. Dopaminergic variations in transmission control the flow of information through the limbic part of the basal ganglia. Therefore, the dopaminergic system is involved in several central functions of the brain including reward, motivation, response to stimuli and motor movement. This has been shown extensively in drug addiction studies where drugs such as psychostimulants interact with neurotransmitters to increase extracellular levels of dopamine. This increase in dopamine appears to be involved in the self-administrative, addictive behavior associated with psychostimulants. These results have been used to hypothesize that the dopaminergic system may play a role in the reward associated with voluntary running (Pierce & Kumaresan, 2006). Meeusen et al. (1997) found that exercise increases the dopamine concentration in the reward center in the brain in rats. The dopaminergic system is complex and regulation of physical activity is thought to be multifaceted. There are variations throughout the system that may regulate physical activity including receptor expression and downstream signaling pathways such as variation in expression of transcription factors and other factors affecting gene expression. We will focus on the regulation of monoamine oxidase-A and the genetic variations within this mechanism.

## Monoamine Oxidase A

MAO-A is an enzyme whose purpose is to degrade neurotransmitters such as dopamine and serotonin (Kalat, 2008). As discussed previously there are genetic variations in the dopaminergic system, one of these variations is a difference in sequence repeats called a variable number of tandem repeats (VNTR) which are associated with different rates of transcription. There are two alleles; the high-efficiency, 4 repeat allele that results in high transcriptional activity of MAO-A and the low–efficiency, 3-repeat allele that results in low transcriptional activity of MAO-A. The VNTR is located on the X chromosome meaning males are homozygous because they only have one copy of the allele and females can be homozygous or heterozygous because they have two copies of the allele. However, it is unclear how MAO-A is affected by random inactivation of one of the X chromosomes in females (Buades-Rotger & Gallardo-Pujol, 2014).

Wayment, Schenk and Sorg (2001) examined the rate of clearance of dopamine in the rat medial prefrontal cortex. They found that when MAO-A was inhibited the dopamine took about 30-50% longer to clear the medial prefrontal cortex than when MAO-A is present and active. This shows that MAO-A is an important enzyme in regulating the concentration of dopamine in the brain.

The transcriptional activity of MAO-A is regulated by a transcription factor, nescient helix-loop-helix 2 (NHLH2). The function of NHLH2 is dependent on acetylation at lysine 49, which allows the transcription factor to bind to the promoter region of its target gene, MAO-A. Genetic variations can alter acetylation of the protein and its activity on target genes.

## MAO-A and Physical Activity

MAO-A and NHLH2 may relate to regulation of physical activity because they affect the concentration of dopamine available in the limbic portion of the basal ganglia in the brain. For instance, high levels of MAO-A would cause increased degradation of dopamine and thus reduced dopamine signaling. It is thought that individuals who possess the high-transcriptional allele are thought to have lower physical activity levels than those who have the low-transcriptional allele (Good et al., 2015; Figure 4). This study showed that there was a positive correlation between MAO-A VNTR genotype and activity level in young girls. They found that girls who had the low-transcriptional 3/3 VNTR were more physically active than girls with the high-transcriptional 4/4 VNTR. They did not find a significant difference between boys activity level and the two genotypes. They concluded this could be due to MAO-A being on the X chromosome. However, it is not clear how expression of MAO-A is affected by X-inactivation in females.

Variations in NHLH2 may also play a role in the regulation of physical activity through regulation of MAO-A and dopamine levels. Libert et al. (2011) found that when NHLH2 was deacetylated mice were less motivated to explore their surroundings than when NHLH2 was acetylated. In this case NHLH2 would bind to the MAO-A promoter and repress MAO-A activity so dopamine levels remain higher and the mice were more motivated to explore. Mice with a targeted deletion of NHLH2 showed that although the mice have normal levels of food intake, their voluntary exercise was decreased by 50% when given access to running wheels (Coyle et al., 2002). NHLH2 variation can lead to decrease in voluntary exercise through changes in transcription of MAO-A. It is hypothesized that individuals with genetic variations leading to low acetylation of NHLH2 and high transcriptional 4/4 repeat VNTR in MAO-A would be expected to show the highest levels of sedentary behavior.

## Rationale

Physical activity is an important aspect a healthy lifestyle. However, the majority of the adult US population does not meet physical activity guidelines set by the CDC. This is in part due to a lack of motivation to participate in physical activity. Individuals may initiate an exercise program to lose weight, however, in order to commit to participating in physical activity for a long period of time, they must be intrinsically motivated (Friederichs et al., 2015). Intrinsic motivation comes from the feeling of internal satisfaction or reward and the desire to meet the basic psychological needs (Teixeira et al., 2012). Dopamine is the neurotransmitter responsible for the central reward system and has been linked to motivation. There are several genetic variations in the dopaminergic system that could regulate the motivation to engage in physical activity. Enzymes including the MAO-A enzyme alter the concentration of dopamine in the synaptic cleft of a neuron. Thus far, research has shown the concentration of the MAO-A enzyme can vary due to genetic variations including the transcription factor NHLH2 and a VNTR in the MAO-A promoter region (Wayment et al., 2001). However, limited studies have examined these genetic variations in humans and the correlation between physical activity level, motivation and genotype. By determining the MAO-A genotype and examining physical activity levels and motivation to perform physical activity, we hope to determine whether variations in the dopamine pathway underlie the biological basis for motivation and physical activity levels.

## Methods

### Participants

Seventy-one participants were recruited from the Linfield College student body (n=13 males and 58 females) by classroom solicitation and a campus wide email. Extra credit was also offered in the nutrition class. Participants were required to be the traditional college student between the ages of 18-24 years. Participants had their height and weight taken in order to calculate body mass index (BMI, $kg/m^2$). The Linfield College Institutional Review Board vetted and approved all methods. All participants gave informed consent.

### Questionnaires

Each participant completed two questionnaires. The presentation order of the questionnaires was varied between participants. The International Physical Activity Questionnaire (IPAQ) was given to determine levels of physical activity. The IPAQ assesses physical activity level based on self-reported activity over the past 7 days. Questions included "how many days in the past seven days did you do the following types of exercise for at least 10 minutes a day?" The three types of exercise were 1) "vigorous physical activities like heavy lifting, digging, aerobics or fast bicycling", 2)"moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis", and 3) walking " this includes at work and at home, walking to travel from place to place and any other walking that you have done solely for recreation, sport, exercise or leisure." The answers for these questions were weighted according to typical metabolic equivalent (MET) ratings and summed. The weightings were 8 for vigorous, 4 for moderate and 3.3 for walking (Craig et al., 2003). From here, individuals were classified into one of three categories based on their physical activity level. Category 1 is the lowest level of physical activity and defines individuals as insufficiently active. Category 2 defines individuals as minimally active and category 3 defines individual's physical activity as health enhancing physical activity.

The Behavioral Regulation in Exercise Questionaire-2 (BREQ-2: Markland & Tobin, 2004) was given to assess motivation to exercise. The BREQ-2 consists of 19 items scored on scale from 0 (not true for me) to 4 (very true for me). Each question was related to a category of motivation as defined by the self-determination theory (Deci & Ryan, 2000); amotivation, external regulation, introjected regulation, identified regulation, and intrinsic regulation. Each category of motivation was assigned a different weight with negative weightings applied to less autonomous regulation and positive weightings applied to more autonomous regulation. The weighting were as follows; amotivation -3, external regulation -2, introjected regulation -1, identified regulation +2, intrinsic regulation +3. The overall BREQ-2 score is representative of the overall autonomy in the regulation of exercise with higher scores indicating higher motivation. In addition, scores for each category of motivation was examined.

## DNA Analysis

**DNA isolation.** DNA was collected and isolated from a cheek cell sample. Participants were instructed to scrape the side of their cheek for 20 seconds, swish

10 ml of saline solution in their mouth for one minute and then spit the solution into a cup. The cheek cells were isolated from the saline solution by centrifuging the samples at 3000 revolutions per minute (rpm) for 10 minutes. The supernatant was discarded and the pellet was transferred to a microfuge tube. InstaGene cell matrix solution (BioRad, 750ul) was added to the microfuge tube and heat shocked at 95°C for 10 minutes then transferred to ice. The solution was then centrifuged again at 14,000 rpm in order to separate the supernatant and pellet. The supernatant contained the DNA and was transferred to a separate microfuge tube. Samples were stored at -20°C until polymerase chain reaction (PCR) analysis.

**Polymerase chain reaction.** The MAO-A VNTR genotyping was carried out using two primers (F: 5′-ACAGCCTGACCGTGGAGAAG-3′ and R: 5′-GAACGGACGCTCCATTCGGA-3′) (Sabol, Hu & Hamer, 1998). PCR reactions contained 5ul of DNA, 25 ul of GoTaq G2 flexi DNA polymerase (Promega, Sunnybale, CA), 0.5 ul of each primer and 19ul of water for a total volume of 50 ul. The PCR program consisted of one cycle of initial denaturation 95°C for 60 seconds, followed by 35 cycles wherein each cycle consisted of denaturation (95°C for 60 seconds), annealing (63.5°C for 60 seconds) and elongation (72°C for 90 seconds). The PCR cycle finished with one cycle of elongation in 72°C for 5 minutes (Nilsson, 2006). Amplification products were separated at 120 V in a 2% agarose gel stained with 5 ul of gel red. Gels were visualized using a ChemiDoc XRS+ system (BioRad, Berkeley, CA). All genotypes were checked independently by two investigators to confirm and validate genotype identification. A random subsample was reanalyzed to verify genotype results.

## Statistical Analysis

Heterozygous individuals were not included in analysis. Only MAO-A 3/3 VNTR and 4/4 VNTR genotype individuals were used for analysis due to the fact that MAO-A is x-linked and the effects of x-inactivation cannot be determined. One-way ANOVAs were used to compare genotypes in the following variables; physical activity level, MET minutes per week, BREQ-2 score, amotivation, external, introjected, identified and intrinsic regulation. BREQ-2 score and categories of motivation was also assessed between the three IPAQ classifications using a one-way ANOVA. A Bonferoni post hoc analysis was used to further conduct pairwise comparisons of group means. All statistical analysis was performed using SPSS. The alpha level for statistical significance was set at $p<0.05$.

# Results

The PCR for the MAO-A genotype revealed three bands at 324 bp, 354 bp and ~394 bp. The 324 bp and 354 bp bands were the expected fragment size as previously reported for this primer set (Sabol et al., 1998). The 394 bp was not expected and was observed only with heterozygous samples (Figure 2). Upon further perusal of previous research papers using this primer set and personal email communication with Dr. Diego Forero (Ojeda et al., 2014), it was discovered that this third band is an artifact of formation of heteroduplex complexes that often occurs with this PCR protocol. The 324 bp and 354 bp fragments were used for genotyping of the seventy-one participants and we were confident in the identification for sixty-five participants. We excluded seven participants due to lack of PCR reaction or unidentifiable bands. The genotype frequencies were 0.246 for 3/3 VNTR, 0.415 for 3/4 VNTR and 0.388 for 4/4 VNTR (Table 1).

A one-way ANOVA was used to compare BREQ-2 scores and specific motivation categories across genotype. The mean scores on the BREQ-2 were not significantly different between genotypes. The external motivation category score was significantly higher ($F(1,34) = 10.142$, $p<0.01$) in the high transcriptional 4/4 VNTR compared to the low transcriptional 3/3 VNTR between genotypes. The other motivation categories were all similar between genotypes (Figure 3).

There were no significant differences in body mass index (BMI) between genotypes ($F(1,36)= 1.124$, $p=0.30$) with values of 24.2 ± 2.1 kg/$m^2$ and 25.9 ± 6.1 kg/$m^2$ of the 3/3 VNTR group and 4/4 VNTR group respectively (Table 2). Both groups border the normal to overweight classification of BMI (American College of Sports Medicine, 2015). Physical activity measured in MET minutes per week was assessed using a one-way ANOVA to compare amount of physical activity across genotype. The mean MET minutes per week was 2637 ± 2207 and 4391 ± 2542 for 3/3 VNTR and 4/4 VNTR respectively (Table 3). There was no significant difference in METs between genotypes.

IPAQ classifies physical activity level in three categories based on MET minutes per week. Sufficiently inactive individuals are classified as category 1, minimally active are classified as category 2 and health enhancing physical activity as category 3. A one-way ANOVA with IPAQ as a between subjects factor was used to compare BREQ-2 scores and categories of motivation across IPAQ classification. There was a significant difference between groups in BREQ-2 score, amotivation, introjected, identified and intrinsic regulation; external regulation was similar between the three IPAQ categories. To assess the differences among IPAQ groups, a Bonferoni post hoc analysis was used. Amotivation was higher in the inactive group than the minimally active or health enhancing groups ($p<0.05$). Identified and internal regulation was higher in the minimally active group than the inactive group ($p<0.05$). Introjected, identified and internal regulation was higher in the health enhancing group than the inactive group ($p<0.05$; Figure 4).

## Discussion

### Genotype and Motivation

The results showed that participants with the low transcriptional (more dopamine) 3/3 VNTR genotype were significantly less externally motivated than the high transcriptional (less dopamine) 4/4 VNTR. This suggests that individuals with the 3/3 VNTR are not as susceptible to influence by external regulators and their physical activity behavior may be more self-determined and autonomous. This relationship may also suggest that those who have the 4/4 VNTR genotype are more likely to participate in physical activity for reasons that do not satisfy basic psychological needs in the context of the self determination theory. For instance they may be susceptible to exercising in order to obtain an award or avoid punishment (Deci & Ryan, 2000).

The results failed to show a relationship between genotype and overall BREQ-2 score, amotivation, introjected, identified and internal motivation. Lack of the expected relationship may be explained by internalization of physical activity behavior. External motivation is on a continuum from external to integrated regulation. The more autonomous a behavior is the more internalized it has become. Physical activity may start as a behavior to avoid gaining weight, however as the individual continues to participate in physical activity they begin to appreciate the values of the behavior, eventually the behavior may become fully internalized and autonomous. When the behavior becomes autonomous it is meeting a basic psychological need and may present as intrinsic motivation even though it began as external (Ingledew & Markland, 2008). Due to the participant's college campus environment and the number of student athletes included in the study, it is reasonable to suggest that participants may have internalized physical activity if it was not initially an intrinsic behavior.

## Genotype and Physical Activity

We predicted that physical activity levels would be higher in the 3/3 VNTR genotype compared to the 4/4 VNTR genotype. Good et al, (2015) found this relationship in young girls, those with the 3/3 VNTR were more physically active than their peers with the 4/4 VNTR. Therefore, it was expected that a similar relationship in a college population would be observed.

It is possible that the large variance in physical activity reported by the IPAQ affected our outcome. Many of the participants were in-season athletes meaning that they were engaged in 2-3 hours of physical activity a day in their sport. Other participants had minimal activity minutes. The variability in physical activity may also in part be due to the nature of self-reporting. Error in self-reporting may have occurred when the participant misunderstood the definitions of moderate and vigorous activity. For instance, a participant played 3 hours of soccer and they recorded 3 hours of vigorous activity. Soccer does involve many quick bursts and sprints. However, they were likely not performing 3 hours of strenuous physical activity; the participant may have been at practice for 3 hours but only 45 minutes was actually spent in rigorous physical activity and the other 2.25 hours was spent recovering, jogging or walking.

Secondly, while genetics is likely to play a role in the regulation of physical activity, the environment also impacts physical activity levels. Moreover, the environment affects most gene expression (Griffiths, Wessler, Carroll, & Doebley, 2015). For our study, participants are immersed in a residential college environment where 77% of the students live on campus, (Linfield College Institutional Fact Book) and every participant has access to the college fitness center. Furthermore, the prevalence of sport and physical activity is high. In fact, 40% of the student population is involved in a collegiate sport. Given this environment, there may be additional pressures from peers to look or act in a certain way. This setting is likely to be a source of external regulation and the internalization of physical activity behavior (Knee & Neighbors, 2002).

## Physical Activity and Motivation

We found that participants with low BREQ-2 scores had lower activity levels as assessed by IPAQ. When BREQ-2 was further broken down into behavior regulation an interesting trend was observed. Individuals in the low activity category had a higher prevalence of amotivation than the two higher activity categories. Those in the higher activity categories also showed greater internal and identified behavior regulation. This pattern indicates that those who are either more intrinsically motivated or have internalized external motivation are more likely to participate in physical activity. Whereas those lacking motivation are going to be less apt to exercise. The insignificant difference in identified and internal regulation between the higher physical activity group suggest that other factors beyond motivation may play a role in physical activity level. One such factor may be sufficient time. A commonly reported reason for not participating in physical activity is not having the time or resources to do so (Teixeira et al., 2011). It should be noted that only two participants were classified in the inactive category. This limits the significance of our outcome but provides impetus for future work.

## Limitations

There are several limitations in the current study that may have influenced the observed results. As mentioned previously, there were some errors encountered in completion of the IPAQ questionnaire. In the future it may be beneficial to give the questionnaire in an interview format. This would reduce the error in self-reporting and provide a more clear picture of activity frequency, intensity, and type. Tracking physical activity with fitness monitors may also be considered in the future to provide as a correlate for survey data. This is becoming more realistic as technology improves and ultimately could be a more accurate way to assess physical activity levels compared to a recall survey.

Another limitation concerns the sample population. A large portion of the participants were student athletes or heavily involved in physical activity. As mentioned previously, these participants had required workouts for their particular sport. Thus, this exercise would be considered involuntary since participants are not doing the physical activity due to their own volition. While they may be internally motivated to do the exercise despite the requirements, there is no way to tell from the data collected. Further assessment of participant motives is needed in these cases. In addition, the majority of our sample population was female (82%). Due to the low number of male participants, we were unable to separate our analysis by gender. Other studies have shown that females with the 4/4 VNTR had lower levels of physical activity compared to 3/3 VNTR. However, no differences were found between genotypes in males (Good et al., 2015). Thus, a more well-rounded representation of the population is needed for future studies.

## Conclusion

In sum, individuals with a high transcriptional 4/4 VNTR genotype report significantly higher scores in external motivation compared to the low transcriptional 3/3 VNTR genotype. Internal motivation scores were similar between the two genotypes. This suggests that individuals with the 4/4 VNTR may be more susceptible to external motivating factors and that physical activity is a behavior that may be internalized. The current study did not show the expected relationship between genotype and physical activity level as there was no difference in physical activity levels between genotypes. An interesting pattern was shown in regards to physical activity and motivation. The two highest activity categories had significantly more internally motivated individuals than the lowest activity level. This suggests that intrinsic factors play an important role in determining physical activity levels.

## Future Directions

Our study sought to examine a college aged sample population. However, our sample lacked true representation of this population; inclusion of more males and non-athletes is critical. In addition, while we intentionally narrowed our focus to the college environment, the examination of other sample populations that are under more diverse environmental pressure may be of interest and would provide more generalizable insight as to the relationship between genetic background and physical activity. Finally, future studies should examine genetic variations in both NHLH2 and MAO-A in order to determine the interaction between these two dopamine regulatory elements and get a better picture of dopamine's role in the regulation of physical activity and motivation to participate in physical activity.

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## Appendix

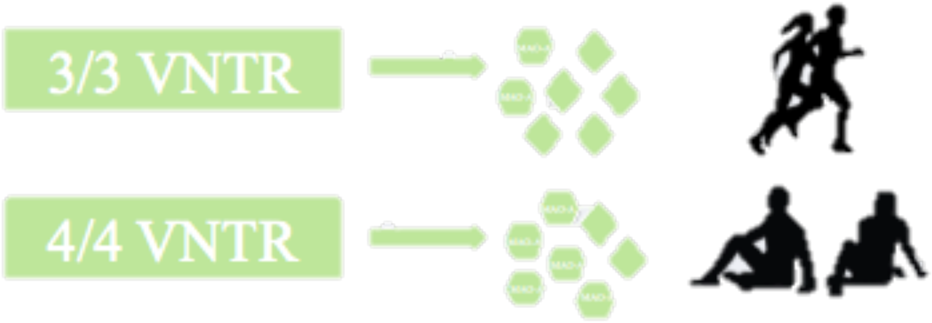


**Figure 1.** Low transcriptional 3/3 VNTR leads to low levels of MAO-A (hexagon shape) and a higher concentration of dopamine (diamond shape). The high transcriptional 4/4 VNTR leads to high levels of MAO-A and therefore lower concentration of dopamine (adapted from Good et al., 2015).

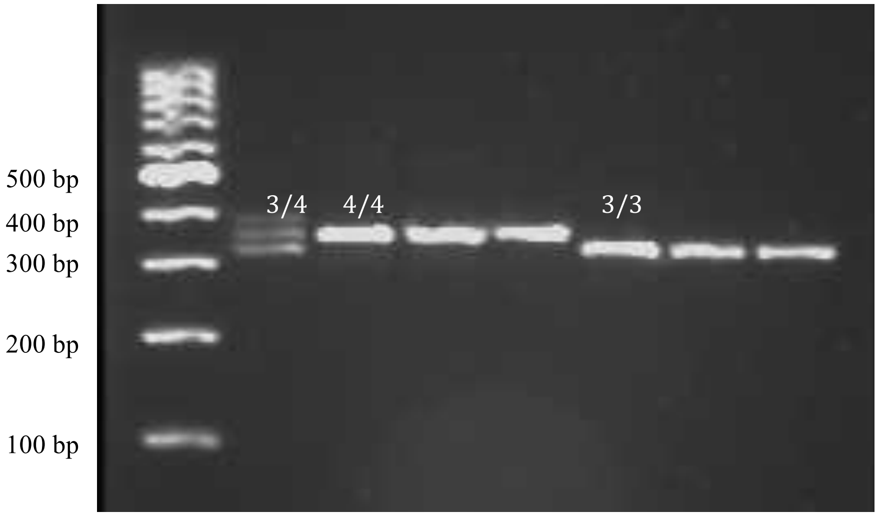


**Figure 2.** A 2% agarose gel showing a 3/4 VNTR genotype with three bands between 300 and 400 bp, a 4/4 VNTR genotype band at 354 bp, and a 3/3 VNTR genotype band at 324 bp

**Table 1:** Genotype Frequencies

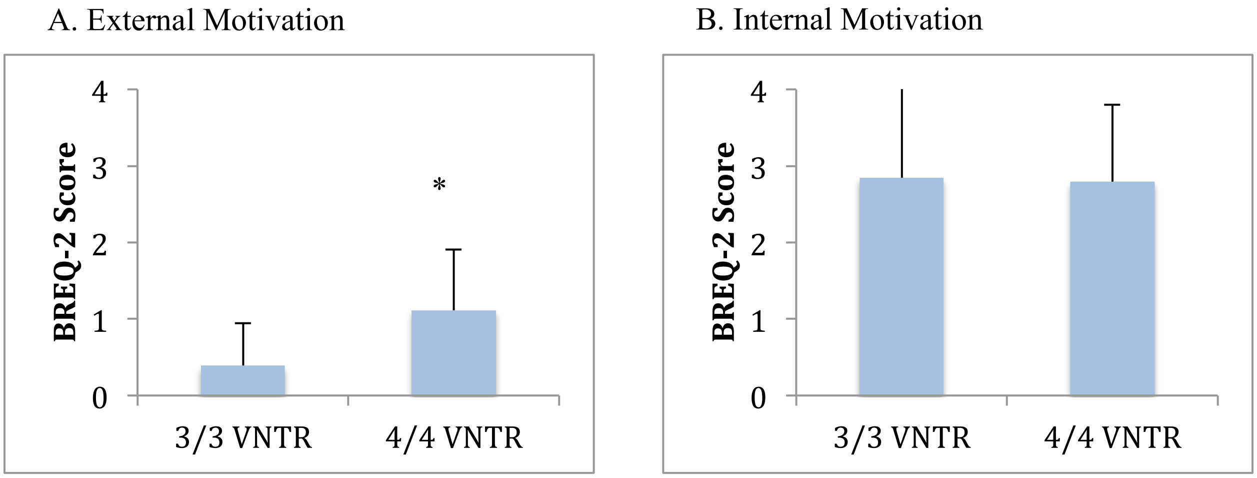
| **Genotype** | **Frequency** |
|---|---|
| 3/3 VNTR | 0.246 (n=16) |
| 4/4 VNTR | 0.388 (n=22) |
| 3/4 VNTR | 0.415 (n=27) |

**Table 2**: Demographics

| **Genotype** | **Age** | **Height (m)** | **Weight (kg)** | **BMI ($kg/m^2$)** |
|---|---|---|---|---|
| 3/3 VNTR | 19.8 ± 1.3 | 1.7 ± 0.1 | 73.0 ± 10.3 | 24.2 ± 2.1 |
| 4/4 VNTR | 19.6 ± 1.4 | 1.7 ± 0.06 | 75.0 ± 18.3 | 25.9 ± 6.1 |

**Table 3:** IPAQ physical activity frequencies

| **Genotype** | **Mean MET minutes per week** | **IPAQ physical activity category** | | |
|---|---|---|---|---|
| | | 1(Low) | 2 (Moderate) | 3 (High) |
| 3/3 VNTR | 2637 ± 2207 | 1 | 7 | 8 |
| 4/4 VNTR | 4391 ± 2542 | 1 | 5 | 16 |


A. External Motivation

B. Internal Motivation

**Figure 3A.** External motivation to exercise scores were significantly higher in the high transcription 4/4 VNTR genotype compared to the low transcription 3/3 VNTR genotype. $*p<0.01$ **B.** Internal motivation to exercise scores were not significantly different between genotypes.

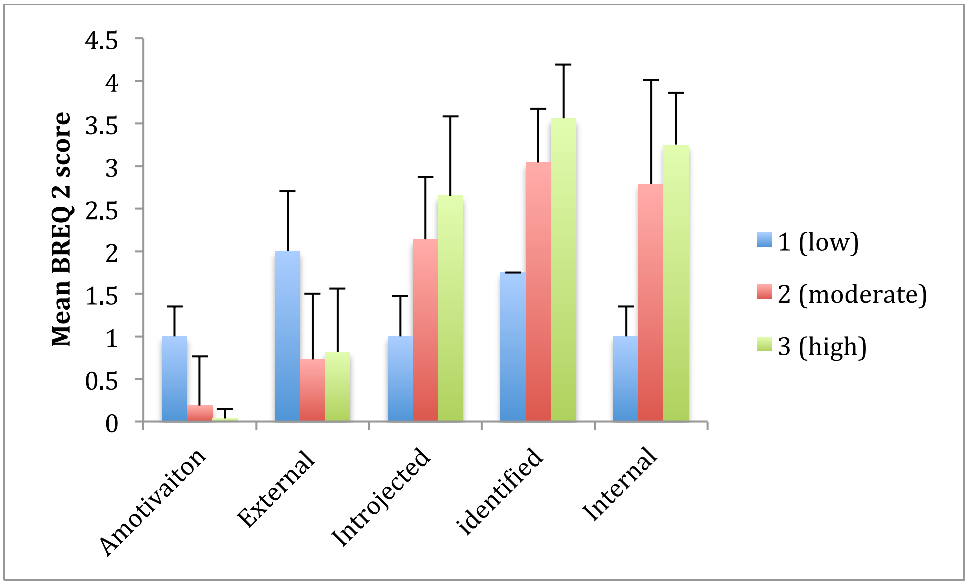


**Figure 4.** Amotivation was higher in the inactive group (1, low) than the minimally active (2, moderate) or health enhancing groups (3, high) ($p<0.05$). Identified and internal regulation was higher in the minimally active group than the inactive group ($p<0.05$). Introjected, identified and internal regulation was higher in the health-enhancing group than the inactive group ($p<0.05$)