THE GENETICS OF SPORTS BEHAVIOUR: THE ROLE OF THE DRD4 GENE IN SENSATION SEEKING IN SKIERS

by

CYNTHIA J. THOMSON

B.Sc. & B.P.H.E., Queen's University, 2003

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Human Kinetics)

THE UNIVERSITY OF BRITISH COLUMBIA
(Vancouver)

August 2008

ABSTRACT

Previous research has shown a large genetic influence over personality traits, especially sensation seeking. One gene thought to influence this behavioural trait is the dopamine-4-receptor gene (*DRD4*), in which variants have been associated with sensation seeking and novelty seeking in some, but not all studies. The inconsistencies between studies may be due to heterogeneity in both the behaviours and the populations being assessed. Some studies included only males and few studies have *a priori* analyzed males and females separately. SS has been associated with high-risk sports, including skiing; however, this is the first study to address the possibility that genetics may play a role in individuals' inclination towards SS in sport. Using the Contextual Sensation Seeking Questionnaire for Skiing (CSSQ-S), developed and validated for this study, and the Zuckerman-Kuhlman Personality Questionnaire (ZKPQ), levels of SS in males and females were analyzed in association with the alleles of a polymorphism in the dopamine-4-receptor, -521 C/T (a C or a T at position -521). Behavioural analysis of skiers (N = 200) revealed a significant correlation (r^2= .506, $p < .001$) between skier behaviour (CSSQ-S) and skier personality score (ZKPQ) for sensation seeking. Genotype analysis (N = 74) revealed allele frequencies of .58 C and .42 T and an over-representation of the C allele was found in the population of skiers compared with a general Caucasian population ($p < .01$). In females, a significant association was found between the homozygous C/C genotype and high levels of contextual skiing SS behaviour (N = 36**,** $p = .006$, $\eta^2 = .2$), along with a non-significant trend between ZKPQ impulsive SS scores and the alleles of -521 C/T ($p = .086$). No association, however, was found in males (N=38, $p_{ZKPQ} = .473$, $p_{CSSQ\text{-}S} = .345$). This study supports the hypothesis that alleles of the *DRD4* -521 C/T polymorphism are associated with context-specific SS behaviours, however only in females. Social pressures may differentially influence male and female sensation-seeking behaviour which may explain the lack of association in males, though this hypothesis requires further investigation.

TABLE OF CONTENTS

APPENDIX P: Cronbach alphas and tests for normality of ZKPQ subscales using total scores from all subjects (N = 201). 80

LIST OF TABLES

LIST OF FIGURES

GLOSSARY

Allele: one version of a genetic variant at a particular polymorphic locus (*e.g.* 'C' or 'T' at a 'C/T' polymorphism).

Bases: the individual nucleotides that comprise DNA (A, C, T and G).

Chromosomes: long segments of DNA on which genes are encoded. Humans have 23 distinct chromosomes and every cell (except sex cells) has two complete sets (one from mother, one from father).

Dopamine: a neurotransmitter in the brain that has both excitatory and inhibitory functions related to motor control, motivation and reward pathways.

DRD4: the dopamine-4-receptor gene, one of five receptor subtypes for dopamine.

Eigenvalue: algebraic transformation variables that represent the variance accounted for by each factor in a factor analysis.

EPI: Eyesenck Personality Inventory measures extraversion/introversion and neurotism.

Factor: items that describe different components of the same larger dimension (*e.g.* a personality trait) comprise a factor. A factor is a single dimension that is independent, but is composed of highly related items.

Factor Analysis: a statistical method used to group items (*e.g.* in a questionnaire) according to their relatedness.

Gene: A protein-encoding segment of DNA. Genes are located on chromosomes.

Genotype: The combination of alleles at a particular locus (*e.g.* C/C, C/T or T/T at a 'C/T' polymorphism).

HPLC: high performance liquid chromatography, a purification method.

Maximum likelihood rotation: a rotation of eigenvalues by 90 degrees, used in factor analysis.

NEO-FFI: NEO Five-Factor model measures five basic dimensions of personality traits including neurotism, extraversion, openness to experience, agreeableness, and conscientiousness.

Neurotransmitter: a brain chemical that transmits signals between neurons.

PCR: polymerase chain reaction, in the context of this study PCR is a method that allows amplification of a specific DNA segment via varied cycled temperatures.

Personality: the consistent pattern of behaviours that is characteristic of an individual. Personality is often organized in conceptual taxonomic structures.

Phenotype: an observable characteristic of an individual that is influenced by genetic and environmental factors.

Polymorphism: a common variation in DNA in which alternate sequences occur in populations.

Polymorphic locus: the location in the DNA of a polymorphism.

RFLP: restriction fragment length polymorphism, a method used to identify polymorphisms using an enzyme that either recognizes a specific allele and cuts at the specific site in the sequence, or does not recognize the allele resulting in an un-cut strand.

SSS-IV, V: Sensation Seeking Scales forms IV and V measures four subscales of sensation seeking: thrill and adventure seeking, experience seeking, boredom susceptibility and disinhibition.

SNP: single nucleotide polymorphism, a variation in the gene sequence that occurs at a single locus (one nucleotide base).

TCI: Temperament and Character Inventory which was developed by Cloninger to measure four temperaments traits: novelty seeking, harm avoidance, reward dependence and persistence.

Trait: a behavioural characteristic of a person that is stable over time.

Varimax rotation: a type of rotation in space that maximizes the variance captured by the items in a factor, used in factor analysis.

VNTR: variable number of tandem repeats occurs when a segment of the gene sequence is repeated a variable number of times.

ZKPQ: Zuckerman-Kuhlman Personality Questionnaire measures five traits: impulsive sensation seeking, aggression-hostility, neurotism-anxiety, sociability and activity.

ACKNOWLEDGEMENTS

First and foremost I would like to thank Jim Rupert for his support and guidance throughout my master's experience. Early in my studies, Jim noticed a trend in my extra-curricular activities and wondered if there might be genetic influences guiding my urges for thrilling activities. Intrigued, I launched into the field of behavioural genetics and I am thrilled that this became the topic of my thesis.

I would also like to thank my committee members, Dr. Mark Beauchamp and Dr. Wendy Robinson for their expertise and support. Mark introduced me to the field of psychology, and was patient and available for extra guidance as psychology was outside my area of study. Similarly, Wendy and members of her lab, especially Courtney Hanna, helped me troubleshoot the genetic analysis, also an area in which I lacked experience. Their patience and generosity of lab materials helped me greatly.

The GRIP lab members at UBC were my support network, giving me advice on genetics and helping me with edits. I would like to thank Pei, Matt, Maria and Mona for their support.

Finally, I would like to thank my friends and family for supporting me through stressful times. My number one supporter, my best-friend and partner, Chris, for joining me on weekend trips to the lab, helping me recruit subjects in Whistler and for being my rock!

INTRODUCTION

Overview

Personality is defined as the consistent pattern of behaviours, often organized in conceptual taxonomic structures, that are characteristic of an individual (Gana and Trouillet, 2003). The factors underlying personality traits are not fully understood. Environmental influences are no doubt of great importance, but the influences of genetics on character are not completely understood and have proven challenging to study. Association studies are a common method for studying the relationship between genetics and personality traits. Personality traits related to the seeking of novel experiences have been extensively studied in association with the genetics of a chemical in the brain called dopamine. The sensation of pleasure (*i.e.* the "high" that a sky-diver might feel during and after a jump) has been hypothesized to be related to the density of dopamine receptors (Franken, Zijlstra, & Muris, 2006). Dopamine levels have been related to motivation and reward mechanisms, which is why dopamine pathways in the brain are thought to be involved in the sensation-seeking trait. Sensation seeking has been associated with behaviours that are deemed high-risk in Western society, including participation in certain types of sports. Traditionally, the study of sensation seeking and sport has been limited to the field of psychology, and this study is the first to investigate the genetics of sensation seeking in a sport cohort. Questionnaires exist to measure global sensation seeking, namely the overall personality trait across all situations, but we developed a sport-specific questionnaire that measures contextual behaviours related to sensation seeking in skiers and snowboarders. This study focuses on the sensation-seeking personality trait, sport-specific behaviours in skiing and snowboarding associated with the trait, and whether an association exists between sensation-seeking levels and variations in the gene encoding one of the dopamine receptors.

Risk-taking and evolution

What propels certain individuals to fearlessly jump out of airplanes and speed down ski runs? Downhill-mountain bikers, skiers, mountaineers and other "extreme" athletes alike, risk all and show no restraint in order to rise to the top of their sport. A

common thread among many high-risk sport athletes is a craving for thrill and adventure (Anonymous, Elevation), but to what degree is it genetically "hard-wired" or influenced by environmental experience? Day to day challenges of many early settlers such as hunting, finding shelter and fighting for ones territory were risky activities (Zuckerman, 2000). In the Western world strict safe-work policies and the amenities of urban life have removed natural outlets for thrill and adventure. Perhaps to fill this void, a new class of recreation involving higher physical risks has emerged, gaining popularity since the 1970s (Creyer, Ross, & Evers, 2003).

Sensation seeking

High-risk sports include "any sport, where one has to accept a possibility of severe injury or death as an inherent part of the activity" (Kajtna, Scaronak, Bari, Cacute, & Burnik, 2004). Ice-hockey for example, is not a high-risk sport, because although bodily harm is common, the potential for death is low. Sky-diving, on the other hand, is an example of a high-risk sport, since the stakes for possible death are greatly increased. Downhill-skiing and snowboarding, the sports highlighted as the focus of this study, are also considered risky activities and their popularity has been increasing over the last few decades (Bouter, Knipschild, Feif & Volovics, 1988).

Certain personalities may be more attracted to risky sports, while others may shy away from these potentially dangerous activities. The personality trait, "sensation seeking", coined by Zuckerman, emerged from observed behavioural differences between individuals coming out of sensory deprivation experiments (Zuckerman, 1979, p. 10). Sensation seeking (SS), involves a desire to seek out new and thrilling experiences, and involves taking risks for the sake of such experiences (Zuckerman, 1979). Risk is a relative term however, and it is important to note that sensation-seeking behaviours involve the risk perceived by the participant, not by the observer.

Sensation seeking and related traits

Sensation seeking is empirically measured using standardized personality questionnaires that have evolved over the years. The Sensation Seeking Scale (SSS) Forms IV and V are questionnaires that have been used in psychology research since the 1970s to measure four subscales of sensation seeking: experience seeking (ES), thrill and

adventure seeking (TAS), boredom susceptibility (BS), and disinhibition (DIS) (Zuckerman, 2006). In 1993, the questionnaire was modernized and expanded to a five-trait measure, becoming the Zuckerman-Kuhlman Personality Questionnaire (ZKPQ). This questionnaire measures impulsive sensation seeking (a combination of impulsivity and sensation seeking, ImpSS), along with four other personality traits: aggression-hostility (Agg-Host), sociability (Soc), neurotism-anxiety (Neur-Anx), and activity (Zuckerman, Joireman, Teta, & Kraft, 1993). Three of the traits, ImpSS, Agg-Hos and Soc were associated with risk-taking behaviours (including smoking, drug-use, and reckless driving) in a study on college students (Zuckerman & Kuhlman, 2000). Both the ZKPQ and the SSS questionnaires have been used to study sensation seeking in association with certain sports (Table 1) and other high-risk activities.

Sensation seeking has often been associated with two other related traits: novelty seeking and extraversion. Novelty seeking has also been studied in association with risky behaviours, however novelty seeking differs in both the instrument of measurement (McCourt, Guerrera & Cutter, 1993; Schweizer, 2004), and its scope. Novelty seeking has a slightly narrower scope than sensation seeking and lacks the facet of risk taking (Zuckerman, 2005). The trait "extraversion" has been included in literature reviews of novelty and sensation seeking (Munafo, Yalcin, Willis-Owen, & Flint, 2008); however, extraversion is a broader personality dimension that includes novelty seeking as one component of a much broader trait characterized by activity, gregariousness, and positivity (Bookman, Taylor, Adams-Campbell, & Kittles, 2002; Ebstein, 2006).

Studies have shown that the three traits: novelty seeking, sensation seeking and extraversion, are related (McCourt et al., 1993; Zuckerman and Cloninger, 1996; Bookman et al., 2002) and each has its own instrument of measure. The SSS and the ZKPQ measure sensation seeking, while Cloninger's Tri-Personality Inventory (TCI) and NEO-five factor inventory are used to measure novelty seeking and extraversion respectively (Cloninger, 1987; Reuter and Hennig, 2005). The ZKPQ was chosen to measure the sensation-seeking trait in this study of skiers (rationale is explained in the discussion). All of the above-mentioned questionnaires measure global personality traits and are not situation specific; therefore, for the purpose of this study a sport-specific

questionnaire was created in order to assess contextual sensation seeking behaviours (See Methodology and Appendix A).

Sensation seeking and sports

The sensation-seeking trait has been associated with high-risk sports and other high-risk social activities including promiscuous sex, illicit drugs, and crime (Gelernter, Kranzler, Coccaro, Siever, New, & Mulgrew, 1997; Zuckerman, 2000; Zuckerman and Kuhlman, 2000; Franques, Auriacombe, Piquernal, Verger, Brisseau-Gimenez, Grabot, & Tignol, 2003; Kelly, Robbins, Martin, Fillmore, Lane, Harrington, & Rush, 2006). Some people may satisfy a need for adventure-seeking through risky sports, while others may be driven to the less socially accepted forms of thrill-seeking. Logically, high-sensation seekers tend to be involved in high-risk sports, and low-sensation seekers in low-risk sport (Table 1). The studies listed below compared sensation-seeking scores between high- and low-risk groups, but did not inquire about specific behaviours associated with the high-risk activity. Few studies to date have measured context-specific high-risk behaviours since most studies have measured sensation-seeking using standardized personality questionnaires that measure traits across all situations.

Table 1: A summary of research relating sensation seeking to high- and low-risk sports.

High-risk (N)	Controls and low-risk (N)	Measure	Findings	Ref.
Hand-gliding, mountaineering, skydiving, auto racing (93)	Swimming, marathon running, aerobics, golf (73)	SSS V	SS higher in high risk group	1
Surfing (41)	Golfing (44)	SSS V	Surfers scored higher on TAS and ES	2
Downhill skiing (219)	Matched reference (299)	SSS	Skiers scored higher on TAS	3
Paragliding (opioid dependent, OD) (34)	College staff (OD) (34)	SSS IV	Para gliders scored higher on TAS and DIS	4

[1)] (Jack and Ronan, 1998); [2)] (Diehm and Armatas, 2004); [3)] (Bouter et al., 1988); [4)] (Franques et al., 2003)

Sensation seeking is also associated with physical risk-taking behaviours in children. Children who were willing to walk on a balance beam set at greater heights scored higher on sensation-seeking scales, the children included in the study were as young as 6 years of age. (Morrongiello and Lasenby, 2006). Perhaps sensation seeking is an inherent trait, independent of experience. Some children may be born with higher levels of sensation seeking than others, suggesting a greater influence from genetics than the environment (*e.g.* a younger child has a shorter exposure to environmental influences) although additional support is required.

Dopamine and a physiological basis for novelty and sensation seeking

Previous research demonstrated that dopamine, a neurotransmitter that acts as a messenger in the brain, is a key molecule in reward and pleasure pathways, therefore becoming an important focus of sensation-seeking studies (Okuyama, Ishiguro, Nankai, Shibuya, Wantanabe, & Arinami, 1999). Neurotransmitters are molecules in the brain that are released by one neuron and taken up by the next neuron in series to transmit signals (Figure 1). The action of a neurotransmitter in the synaptic cleft can either increase or decrease the action of a messenger inside the next neuron, thereby triggering (or inhibiting) a cascade of intracellular effects.

Cloninger (1987) theorized that each neurotransmitter may be associated with a broad personality trait, and suggested that dopamine is involved in the novelty-seeking trait. Dopaminergic neurons are activated when an unexpected reward is presented, which is why they are thought to be involved in the learning process (Pecina, Cagniard, Berridge, Aldridge, & Zhuang, 2003). Further, animal models have shown that exposure to a novel environment elicits an increase in dopamine release (Rebec, Christensen, Guerra, & Bardo, 1997).

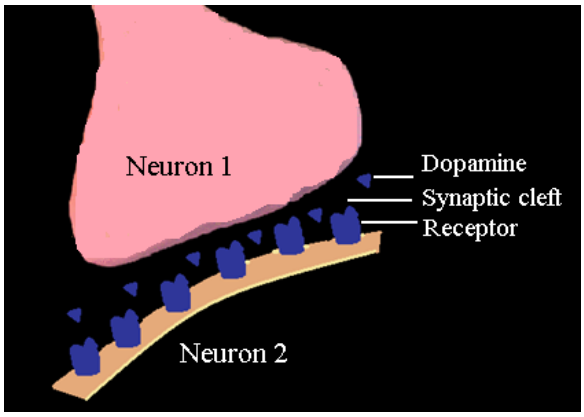


Figure 1: Neurotransmission: dopamine and its receptor in the synaptic cleft.

An excess of dopamine lingering in the synaptic cleft may be responsible for a "high" sensation, similar to the mechanism of action for cocaine, while an exaggerated uptake of dopamine may result in a lack of the ability to feel pleasure, or "anhedonia" (Franken et al., 2006). Variations in the receptors that take up dopamine may affect sensations of reward. Individuals who experience anhedonia in the face of normal stimulation might constantly seek increased levels of stimuli (*e.g.* speed, height, fearful situations) in order to experience some feeling of reward. On a physiological level, a person with a modified receptor might exhibit an altered (blunted or increased) uptake of dopamine, thus modifying his/her sensation of reward and affecting the motivation to repeat an action (Pain, 2005). A simplified conceptualization used in biochemistry is the 'lock and key' model: some individuals have a configuration where the lock matches the key, while others have a lock that does not quite match the key, making the union less successful (Figure 2).

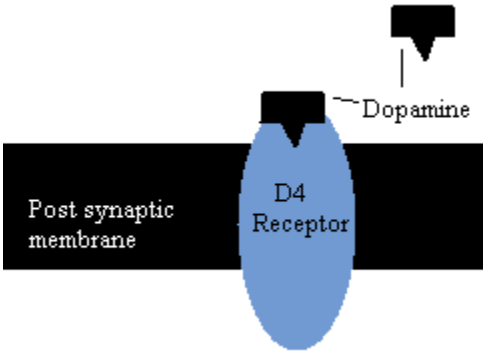


Figure 2: Lock and key model. Dopamine is shaped to fit into its designated receptor.

There are five dopamine receptor subtypes (D1 to D5) in the human brain to which dopamine may bind. The D4 is the receptor of interest for this study due to its primary association with novelty seeking in humans (Benjamin, Patterson, Greenberg, Murphy, & Hamer, 1996; Ebstein, Novick, Umanky, Priel, Osher, Blaine, Bennett, Nernanov, Katz, & Belmaker, 1996; Okuyama et al., 2000; Ronai, Szekely, Nermoda, Lakatos, Gervai, Staub, & Sasvari-Szekely, 2001; reviewed by Munafo et al., 2008 and Schinka, Letsch, & Crawford, 2002). The D4 receptor belongs to the 'D2-like class' of receptors (named after the D2 receptor sub-type), which are inhibitory, causing a decrease in adenylyl cyclase, a molecule involved in neuron-to-neuron communication (Strange, 2000). The D4 is found mainly in the prefrontal cortex, hippocampus, thalamus and hypothalamus; regions of the brain that are part of the limbic system. The limbic system is primarily involved in emotion and memory and because the D4 receptors are so densely distributed in these regions, a role for D4 in attention, motivation and memory has been hypothesized (Kreek, Nielson, Butelman, & LaForge, 2005; Mitsuyasu, Hirato, Sakai, Shibata, Takeda, Ninomiya, Kawasaki, Tashiro, & Fukymaki, 2001).

Pecina and colleagues (2003) measured the behaviour of hyper-dopaminergic mice in response to a reward (a sweet treat) and observed that the mutant mice showed higher wanting, or motivation, towards the reward. This may be related to drug-addict behaviour, in that certain individuals may be predisposed to addiction because they have a continuous wanting of the drug due to sensitization of limbic pathways (Pecina, 2003). Similarly, in the sport setting, certain individuals may have an insatiable urge for increasingly thrilling activities.

Further support for the role of dopamine in the sensation-seeking trait may be inferred from the relationship between dopamine and monoamine-oxidase type-B (MAOB). MAOB is an enzyme that breaks down dopamine in the synaptic cleft. MAOB is higher in females and increases with age; the trends are inversely related to sensation seeking, which is lower in females and decreases with age (Zuckerman, 2000; Zuckerman and Kuhlman, 2000).

Inheritance and genetics of sensation seeking

Identical twins are useful candidates for studying the inheritance of physical and psychological traits because monozygotic twins are genetically identical. Variations in individual characteristics, or phenotypes, are due to a combination of environmental and genetic influences: Variability in phenotype (V_P) = variability due to environment (V_E) + variability due to genetics (V_G), in identical twins $V_G = 0$. The heredity of a trait may therefore be determined by analyzing trait scores in monozygotic twins, and any variation that exists is assumed to be due to the environment (since $V_G = 0$). The greater the environmental influence, the lower the inheritance of the trait.

Twin studies have shown that personality traits have a genetic basis for inheritance, and sensation seeking has been reported to be one of the mostly highly inherited traits (Koopmans, Boomsma, Heath, & van Doornen, 1995; Stoel, De Geus, & Van Tol, 2006). Complex personality traits follow a pattern of quantitative inheritance (Golimbet, Gritsenko, Alfimova, & Ebstein, 2005), meaning that the sensation-seeking trait likely involves the interaction of numerous genes. A Finnish study analyzed both childhood rearing and genetic make-up and found that the genetic influence plays a dominant role in the novelty-seeking trait (Keltikangas-Jarvinen, Raikkonen, Ekelund, & Peltonen, 2004). Large-scale twin studies have used Zuckerman's questionnaires to compare levels of sensation seeking between twin-pairs reared separately (thereby differing in their environmental influences) and also between twin-pairs and their non-twin siblings sharing the same environment. While some variation is due to environment, as high as 60% of the variation in sensation-seeking traits may be explained by genetics (Hur and Bouchard, 1997; Stoel et al., 2006).

There exist sex differences in the source of variation. Sensation seeking in females may be more influenced by their genes, with zero variation being due to a shared environment, where as sensation seeking in males may be influenced by a combination of genes and environment (Stoel et al., 2006). Bookman and colleagues (2002) found a stronger association with extraversion and the -521 T variant of the D4 receptor gene in females, and other studies that included females showed similar trends (Lee, Kim, Kim, Kim, Lee, Joe, Jung, Suh, & Kim, 2003; Ono, Manki, Yoshimura, Muramatsu, Mizushima, Higuchi, Yagi, Kanba, & Asai, 1997).

The molecular biology of novelty and sensation-seeking behaviour

Humans have two sets of 23 chromosomes that carry genetic information for proteins contributing to the anatomy and function of the body. One set of chromosomes is passed to the offspring from each its mother and father. The chromosomes carry approximately twenty-five thousand protein-encoding entities called "genes" (Consortium, 2004). At the DNA level, humans are 99.9 % the same (Collins and Mansoura, 2001), and variations that exist between individuals are in part due to the relatively small, .1%, differences in our genome. Variations within the genome that are common in a population are called "polymorphisms", whereas new variations are referred to as "mutations". Specific polymorphisms are often chosen as candidates for association studies because the variant may have a causal role in the expression of the phenotype or is associated with a nearby causal variant (Cordell and Clayton, 2005). The polymorphism that is the focus of this study is called a single-nucleotide polymorphism or SNP, in which there are two possible variants (nucleotide base pairs) called "alleles". SNPs are often named for their position in the gene, followed by the first letter of each allele.

Intra-individual differences in the dopamine receptor may be due to variations in the gene responsible for its encoding, the DRD4 gene, located on chromosome 11. The DRD4 gene is highly polymorphic (Oak, Oldenhof, & Van Tol, 2000; Okuyama et al., 2000) which means that at multiple sites in the gene there are variations in the nucleotide sequence that are common in most populations. Two particular genetic variations have been the focus of novelty-seeking association studies: -521C/T and the exon III VNTR.

A SNP in the promoter region of the DRD4 gene, -521 C/T (a cytosine to thymine transition 521 bases upstream from the protein-coding start-site, see Figure 4), is common in a variety of populations (Table 2). Because each individual has two sets of chromosomes, three combinations of nucleotide pairs ("genotypes") at location –521 are possible: C/C, C/T or T/T. The ancestral genotype (based chimpanzees) contained a T allele at position -521 (UCSC Genome Bioinformatics, 2008).

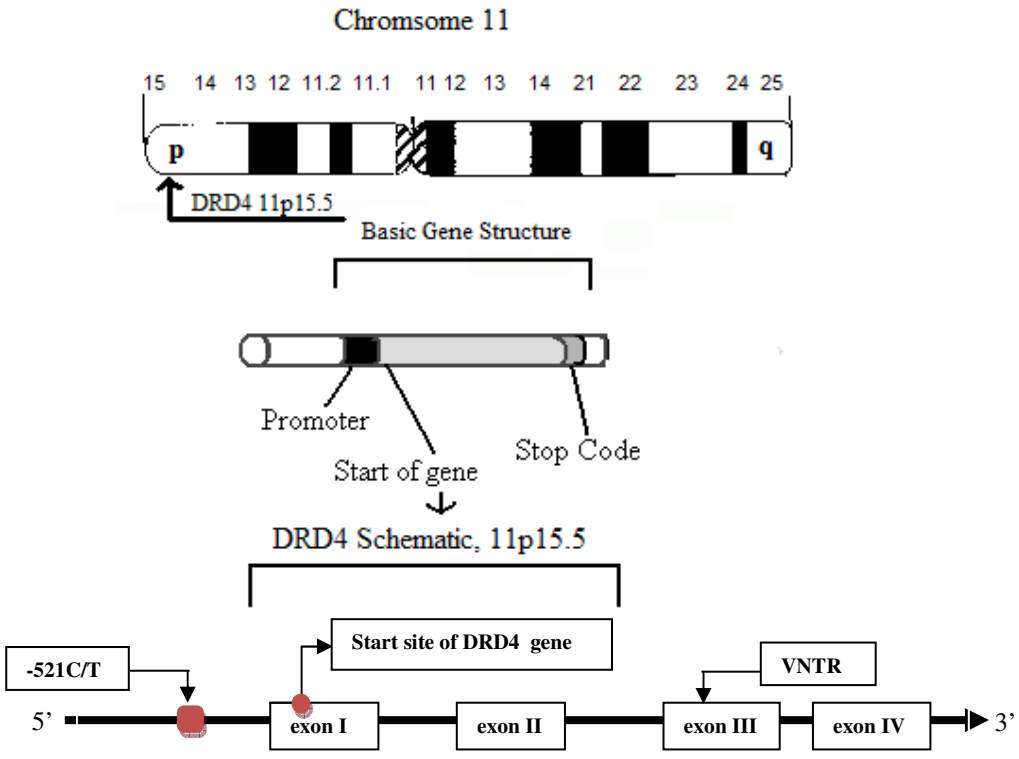


Figure 3: A schematic diagram of the dopamine DRD4-gene (chromosome 11p15.5, NCBI dbSNP number is RS 1800955).
Note: two polymorphisms, [1)] -521 C/T and [2)] VNTR have been studied in association with novelty, sensation seeking and extraversion.

Table 2: -521 C/T allele frequencies across populations.

Origin of population	2N‡	Allele frequency	
		C	T
Swedish (Jonsson et al., 2002)	776	.42	.58
Japanese (Okuyuma et al., 2000)	172	.53	.47
African American (Bookman et al., 2002)	142	.55	.45
Russian (Golimbet et al., 2005)	440	.43	.57

‡2N – 'N' represents the total number of subjects, and two alleles per subject are included in the analysis (2N).

Individuals with the C/C genotype at position –521 have been associated with high novelty-seeking scores (Okuyama et al., 2000; Ronai et al., 2001). Bookman and colleagues (2002) found an association between the T allele and low levels of extraversion, a trait that is related to novelty and sensation seeking (Bookman et al., 2002; Daitzman and Zuckerman, 1980), in females, but not in males. It is important to note that although novelty seeking and sensation seeking are not identical traits, they are thought to share common biochemical pathways (Zuckerman & Kuhlman, 2000). To date, no studies have examined the -521 C/T polymorphism in association with sensation seeking, but based on previous associations between novelty seeking and -521 C/T and the similarities between the two traits, a similar association may be found between the DRD4 gene and the sensation-seeking trait. Overall, seven studies to date have investigated the association between novelty seeking and the -521 C/T SNP (Table 3). Only two studies report a significant association, however, the studies that had non-significant results ($p > .05$) showed a consistent trend of higher novelty-seeking scores in C/C homozygotes compared to the C/T heterozygotes and the T/T homozygotes (Jonsson et al., 2002; Mitsuyasu et al., 2001).

Table 3: Genetic association studies on -521 C/T and novelty seeking and extraversion.

Cohort	N	Measure	Findings	Reference
Young Male	86	TCI	C/C highest NS scores, T/T lowest ($p < 0.001$)*	Okuyama, et al. 2000
Extremes (high and low NS scores)	200	TCI	No significant association	Ekelund, et al. 2001
Schizophrenics and controls	173	TCI	No significant association*	Mitsuyasu, et al. 2001
Students	99	TCI	C/C higher NS than C/T and T/T especially in women ($p = 0.008$)*	Ronai, et al. 2001
Normals	381	TCI	No significant association*	Jonsson, et al. 2002
Normals	276	TCI	No significant association	Strobel, et al. 2002
Adolescent females	101	TCI	No association for -521, but a significant interaction between -521, VNTR and NS	Lee et al. 2003
Normals	104	NEO-FFI	No significant association	Eichhammer, et al. 2005
Normals	220	EPI▲	A joint contribution of –521 C/T and –809 A/G to levels of extraversion	Golimbet, et al. 2005

(*) studies included in meta-analysis by Schinka et al., 2002. (▲) EPI is a measure of extraversion, see glossary (p. ix) for details on the measures.

In simple Mendelian inheritance, an allele may be dominant, recessive, or co-dominant. A dominant allele paired with a recessive allele overshadows the effect of the latter. The inheritance of eye colour in humans is a classic (albeit simplified) example of the Mendelian model: the allele for brown eyes (B) is dominant and the allele for blue eyes (b) is recessive. In order for an individual to have blue eyes, both parents must contribute a 'b' allele to the offspring in order to produce the 'blue' phenotype from the 'bb' genotype. The other combinations of genotypes (BB and Bb) will both result in brown eyes. A pattern of inheritance for the -521C/T SNP has not yet been determined, and dominance of one allele over the other has been inconsistently reported (Mitsuyasu et al., 2001; Ronai et al., 2001). Three studies follow the trend of -521 T as dominant, set by Bookman and colleagues (2002) (Eichhammer, Sand, Stoertebecker, Langguth, Zowe, & Hajak, 2005; Munafo et al., 2008), while others have treated -521 C as the dominant allele (Okuyama et al., 2000; Mitsuyasu et al., 2001; Jonsson et al., 2002).

Only two studies to date have investigated the functional differences between the alleles of the -521 C/T polymorphism (the second was an attempted replication of the first). Constructs containing -521C and –521T alleles were introduced *in vitro* into human cell lines. In the first study, those cells containing the 'C' version of the allele were 40% more transcriptionally active[1] than those with a 'T' allele (Okuyama et al., 2000). The second study however found no significant difference between the two cell-lines (Kereszturi, Kiraly, Barta, Molnar, Sasvari-Szekely, & Caspo, 2006). An up- or down-regulation of receptors may affect personality more than a change in protein structure (Seamen, Fisher, Chang, & Kidd, 1999), this theory is based on the higher density (six-fold) of D4 receptors in the brains of schizophrenics post-mortem (Seeman, Guan, & Van Tol, 1993). The density of receptors is a function of regulation, for example an up-regulation of a gene that translates receptor proteins may result in more receptors. The –521 polymorphism occurs in the 5'(upstream) promoter region of the gene, and while upstream SNPs do not alter the protein itself, they may change expression levels (or regulation) of receptors, which can have major consequences in neuromodulation (Cordell and Clayton, 2005).

A meta-analysis of studies that have investigated the –521 C/T polymorphism in association with novelty-seeking found a small to medium effect size of .32 for the SNP (See (*) in Table 3) (Schinka et al., 2002). A more recent meta-analysis that included studies on extraversion and novelty seeking combined (totalling 11 studies), found a statistically significant effect size of 0.25 and suggested the -521 C/T SNP accounts for up to 3% of phenotypic variance in these related personality traits (Munafo et al., 2008). The proportion of phenotypic variance accounted for by the –521 C/T appears small, however this is consistent with other studies that have reported significant effects (Cloninger, 1987; Reuter and Hennig, 2005). It is important to note that sensation/novelty seeking are complex traits that are influenced by a combination of numerous genes (and within these genes possibly numerous polymorphisms); in addition, environmental influences account for at least 40 % of the phenotypic variance.

[1] This refers to the rate at which the RNA molecules are derived from DNA.

The first polymorphism that was studied in association with novelty seeking is a repeated section of the third coding region of the gene, in which a variable number (2-11) of tandem repeats (VNTR) exist in humans (Van Tol, Wu, Guan, Ohara, Bunzow, Civelli, Kennedy, Seeman, Niznik, & Jovanovic, 1992). Associations were found between subjects carrying seven repeats and high novelty-seeking scores (Benjamin et al., 1996; Ebstein et al., 1996), although replications of these findings have been inconsistent (reviewed by Kluger, Siegfried, & Ebstein, 2002 and Munafo et al., 2008). One hypothesis suggested that variations in the length of the DRD4 gene may cause differences in G-protein coupling and receptor affinity based on the observation that shorter VNTR forms (≤5 repeats) showed different binding patterns with dopamine antagonists compared to the longer (>5 repeats) VNTR forms (Van Tol et al., 1992). Further attempts to characterize the functional differences between VNTR forms found only small differences in binding profiles between antagonists and variants, and the influence of the VNTR may only be minor (Asghari, Schoots, van Kaats, Ohara, Jovanovic, Guan, Bunzow, Petronis, & Van Tol, 1994).

Weak associations with novelty seeking between both the VNTR and the polymorphism at –521 have been found, but the strength of the associations is not sufficient to explain the total variance due to genetics based on twin studies (Noble, Ozkaragoz, Ritchie, Zhang, Belin, & Sparkes, 1998). Likely, multiple genetic variations (and a combination thereof) are responsible for contributing to the novelty-seeking trait (Golimbet et al., 2005), and the same may be true of the sensation-seeking trait. The alleles of the two aforementioned polymorphisms have been found to exist in linkage disequilibrium in some (Ekelund et al., 1999; Strobel et al., 2002) but not all (Jonsson et al., 2002) studies. Linkage disequilibrium (LD) occurs because proximally located variations have a low probability of being separated during DNA recombination and therefore a high probability of being inherited together (Bouchard, Blair, & Haskell, 2007). Inconsistent replications of dopamine-receptor associations may have occurred because the VNTR polymorphism may not directly influence novelty and sensation seeking, but may be in linkage disequilibrium with another polymorphism (*e.g.* –521 C/T) in the gene that may contribute to the sensation-seeking trait.

This study will be the first to correlate sensation-seeking behaviours to genetics in a specific athletic population. Although candidate-gene association studies do not provide causal evidence, they provide a foundation (*e.g.* physiological pathways) for an experimental design that may lead to evidence for a cause-effect relationship. Genetic associations studies are important for demonstrating how specific genetic variations contribute to human characteristics (Cordell and Clayton, 2005). Previous research has identified polymorphisms (VNTR and –521 C/T) that may predispose an individual to novelty-seeking behaviours; however research has focused on drug addicts, schizophrenics or general (predominantly male) populations.

Justification for research

The discovery of a genetic predisposition to the seeking of novel, thrilling and adventurous behaviour is important, because these individuals are often associated with other deviant behaviours including drug abuse, gambling, risky sexual practices and reckless driving (Zuckerman, 2000). With an understanding of the genetic influence on behaviours educators may target such individuals early, encouraging them to participate in thrilling sports as a diversion from other risky behaviour.

Previous studies have measured personality traits using questionnaires that measure personality traits across all situations. A context-specific sensation-seeking questionnaire was created because it may be interesting to test whether global sensation-seeking measures relate to specific behaviours in a high-risk sport and this new questionnaire may provide an additional means of phenotype identification. Neurotransmitters such as dopamine are hypothesized to govern particular reactive behaviours (Zuckerman, 2000) and specific behaviours, as opposed to global traits, may be more directly linked to physiological variations.

Further research into the genetic determinants of sensation-seeking behaviour is needed based on both the highly polymorphic nature of the DRD4 gene and twin studies that have suggested large genetic influences over the sensation-seeking construct. Numerous polymorphisms within the DRD4 gene have been identified; however, previous studies have focused on related traits including novelty seeking and extraversion, and have not explored the genetics of the sensation-seeking trait.

PURPOSE

The purpose of this study was to determine whether an association exists between the upstream polymorphism at position –521 C/T in the DRD4 gene and sensation seeking in skiers and snowboarders.

RESEARCH QUESTIONS

1) Are ski-specific sensation-seeking behaviours associated with ZKPQ global sensation-seeking scores?

2) Is there an association between genetics and sensation-seeking levels among high-risk sport participants?

HYPOTHESES

Main effects hypotheses for the study include:

1. High scores on the ZKPQ sensation seeking subscale will correlate moderately with high scores on the ski-specific behaviour questionnaire (CSSQ-S).
2. Having a C/C genotype at position -521 will be associated with higher scores for sensation seeking.
    a. Overall, there will be a higher proportion of skiers/snowboarders who report high sensation seeking qualities compared with a general population.
    b. The frequency of the 'C' allele will be higher in the population of skiers and snowboarders when compared to the background frequency of the general population.

Interaction hypothesis:

Previous studies have shown a greater variance explained by genetics (as opposed to environment) in females (Bookman et al., 2002; Stoel et al., 2006). The presence of a C/C genotype may have a stronger influence on the sensation-seeking levels of females, resulting in a stronger association in females.

METHODOLOGY

Overview

This was a candidate-gene association study between the alleles of the –521 C/T single nucleotide polymorphism and the sensation-seeking personality trait. Global sensation seeking was measured using the ZKPQ, and context-specific sensation-seeking for skiing was measured using the CSSQ-S. Factor analysis of the CSSQ-S was employed and the degree of association between the two sensation-seeking questionnaires was measured before the genetic association analysis was carried out.

Questionnaire development

A ski/snowboard-specific sensation-seeking questionnaire was developed for the purpose of this study. A focus group (N = 4) of male and female, advanced and expert skiers and snowboarders, ages 25-29 were interviewed to create a list of 'risky' and sensation-seeking behaviours specific to skiing and snowboarding. The definition for the sensation-seeking trait according to Zuckerman (1979) was read to the group members and they were asked to discuss what behaviours in skiing or snowboarding might be carried out by high-sensation seekers. The list initially contained approximately 16 items. Dr. Mark Beauchamp (School of Human Kinetics) and I reviewed the list and items diverging from the definition for sensation seeking along with redundant items were removed, leaving 13 items in the first version of the ski-specific sensation-seeking questionnaire. For the purpose of this thesis, the ski-specific questionnaire is referred to as the Contextual Sensation Seeking Questionnaire for Skiing (CSSQ-S). Examples of the items in the CSSQ-S included, "I like to ski fast" or "I like to go down runs that I have never been down before", scored using a Likert Scale as follows: strongly disagree (=1), disagree (=2), neutral (=3), agree (=4), and strongly agree (=5), indicating the extent to which the subject agreed or disagreed with each statement. The full questionnaire is attached (Appendix A).

The validated Zuckerman-Kuhlman Personality Questionnaire was chosen for measuring levels of global sensation seeking. Permission was obtained from Dr.

Kuhlman, and he included the raw data and full questionnaire from a 1993 publication (Zuckerman et al., 1993) (Appendix B).

Ethical approval was obtained from the University of British Columbia Clinical Research Ethics Board (CREB) in April 2007 and data collection commenced immediately following (Appendix C).

Subjects

Intermediate (and more experienced) level skiers and snowboarders (N = 201, average age = 27.1 years, SD = 4.8)[2], 50 % of whom were women, were recruited from Whistler, British Columbia and Lake Louise, Alberta ski villages. Posters were also displayed around UBC campus, at Mountain Equipment Co-op (a large Outdoor Recreation Co-Operative Franchise), and at fitness centres around Vancouver, British Columbia. Intermediate ability was defined as being able to ski a 'blue square' run comfortably. For the ease of discussion I will refer to the sport of 'skiing' as encompassing both skiing and snowboarding.

Data collection

Study packages were created to accommodate interested subjects who preferred to complete the process on their own time. A consistent ordering of the packages was maintained as follows: consent form, the CSSQ-S (with participant demographics at the beginning), ZKPQ, a consent that explained the peer review process, a peer review questionnaire, and finally an envelope that contained a swab for DNA sampling (Appendices D, E, F). Each portion of the study package is described in detail below. In cases where the subject was able to complete the questionnaire at recruitment time, I administered the package in the same order, and offered only an explanation about how to effectively swab the cheek to obtain cells for DNA. Numerous study packages were handed out with self-addressed stamps, however approximately 30% were not returned.

[2] The ages were based on age groups (e.g. 19-24 yrs), non-continuous variables.

Questionnaire component

The subjects filled out two questionnaires, and a peer of the subject (if available) was invited to fill out a third party questionnaire (a consensual validity check). The first, the Contextual Sensation Seeking Questionnaire for Skiing, CSSQ-S, was a measure of 'context-specific' sensation seeking in skiing or snowboarding that included the dimension of risk-taking (Appendix A). This questionnaire also included a brief demographic section about the subject, including age, sex, race, level of ability and frequency of sporting activity. Race categories were included because frequencies of genetic variants differ between populations (based on HapMap[3]). There were 13 questions to gauge sensation-seeking behaviour in skiing and snowboarding, answered using a Likert scale from 1 to 5. Questionnaire development is described above.

The second questionnaire is a standardized personality questionnaire, the Zuckerman-Kuhlman Personality Questionnaire that assesses five subscales of personality (Zuckerman, 1993) (Appendix B). These include impulsive-sensation-seeking, aggression/hostility, sociability, neurotism/anxiety, and activity. The impulsive-sensation-seeking (ImpSS) subscale was our 'global' measure for sensation-seeking (as opposed to the 'contextual' measure from the CSSQ-S). The ZKPQ inventory includes 99 true or false statements, and there are 19 items in the ImpSS subscale. A sixth subscale, the infrequency scale, includes items that are socially desirable, but are unlikely to be true for anyone (*e.g.* a high infrequency score would be suspect).

Consensual validity check

In an attempt to maintain high consensual validity (which would contribute to the internal validity of the design (Goma-i-Freixanet, Wismeijer, & Valero, 2005), the subject was given the option to have a peer fill out the ski-specific questionnaire about his/her behaviour on the slopes. The measurement of personality traits via questionnaires such as the ZKPQ and CSSQ-S are based on self-report, and the inclusion of a third party allows for the accuracy of the subjects' responses to be verified (Goma-i-Freixanet et al., 2005). It was not, however, an essential component and when it was not possible to find

[3] HapMap is an online database of SNPs in linkage disequilibrium.

a peer who had frequently skied with the individual, this was not included in the evaluation. Discrepancies between the CSSQ-S and the peer review were evaluated using a one-way ANOVA between subject-item scores and peer-item scores.

Genetics

DNA sampling

After completing the questionnaires, the subjects provided buccal (cheek cell) samples by brushing the inside of their mouths with a 'cytobrush' (Medscand Medical AB, Sweden). The samples were stored in paper envelopes at room temperature, allowing them to air dry, and were then frozen at -20˚C for longer-term storage.

DNA isolation

DNA from cheek cells was isolated from the cytobrushes using techniques described by Saftlas, Waldschmidt, Logsden-Sackett, Triche, & Field, (2004). The brushes were incubated at 55°C overnight (at least 8 hrs) in a 700 µL mixture of lysis buffer (Recipes, Appendix G) and proteinease K (.11 mg/mL) to breakdown cellular proteins and remove the cells from the bristles. After incubation, the tubes were centrifuged for 2 minutes at 15 900 g at 4 °C. The brushes were discarded, and RNAse (.03 mg/mL) was added to the supernatant which was then incubated for 60 minutes at 55°C to denature RNA. 320 µL of 5M potassium acetate precipitation buffer (KOAc) was added and the tubes were stored on ice for 10 minutes and then centrifuged (15 900 g) for 5 minutes. The supernatants were transferred to new tubes and the pellets that had precipitated out of solution were discarded. Glycogen (.025 mg/mL) and 510 µL of isopropanol were added to the solution and the tubes were stored on ice for 20 minutes, glycogen acts as a DNA carrier to pull the DNA out of solution (as described in the Invitrogen Catalogue, 2008). The tubes were centrifuged (15 900 g) for 10 minutes and the supernatants were discarded leaving DNA pellets. The DNA pellets were rinsed with a 70% ethanol (200 µL) followed by a 1 minute centrifuge to remove remaining salts. The ethanol was carefully discarded from each tube and the DNA pellets were air dried and re-suspended in 90 µL TE buffer (10 mM Tris/Cl, 1 mM EDTA pH 8.0) for future use.

DNA quantification

The concentration of buccal DNA was analyzed using a spectrophotometer. The machine was calibrated to zero absorbance using samples of distilled water and TE buffer. The absorbance of a 1 μL aliquot of each sample was analyzed at two wavelengths (260 nm and 280 nm) and the concentration of DNA was calculated based on the constant: 1 OD (optic distance) = 50 ug/mL of DNA.

Genotyping by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP)

DNA was amplified using primers specific to the DRD4 gene based on procedures used by Okuyama et al., (2000) with modifications described below, including the use of *Taq* DNA polymerase (Invitrogen, California) instead of *Pfu* polymerase. Primers were chosen to match the genetic sequence surrounding the –521 C/T polymorphism. Forward and reverse primers were chosen based on the literature and the DNA sequence data in HapMap. Once the optimal PCR conditions were established and the primer pair successfully amplified the correct region of the gene the following primer pair was used for the majority of the analyses: the forward primer, F3, 5' - CGG GGG CTG AGG GCC AGA GGC T –3' (T_m = 70.5°C), and the reverse primer, R3, 5' - GCA TCG ACG CCA GCG CCA TCC TAC C – 3' (T_m = 67.5°C) (NAPS IDT, UBC, Vancouver). These two primers matched primers used by Okuyuma and colleagues (2000), with the exception of three base pairs within the forward primer (highlighted above), modified to match the genomic sequence (according to Hap Map, rs1800955). Polymorphisms exist at these three loci (Ronai, Szantai, Nemoda, Lakatos, Gervai, Guttman, & Sasvari-Szekely, 2004), which may explain the discrepancies between primer pairs used in various studies. Two other primers, F4/R4 were used to amplify a portion of the samples prior to the optimizing of the F3/R3 pair. F4 (5' – AGG ATC AAC TGT GCA ACG GGT – 3', T_m = 59.2 °C) and R4 (5' – AGA CGC AGA AAG ACC TGA GC – 3', T_m = 57.2 °C) were created based on the genetic sequence (NAPS IDT, UBC, Vancouver). Since the F3 pair was used for the majority of the analysis, the optimizing conditions for F3 are included in this section, please refer to Appendix H for details on F4/R4.

The optimal PCR condition started with a high heat treatment for a longer duration to denature the GC-rich DNA strand. The final PCR cycled as follows: 98°C for 2 minutes, followed by 39 cycles of 96°C for 45s, 62°C for 45s and 72°C for 2 minutes, finally a chase for 10 minutes at 72°C. The 25 µL reactions contained 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$, 0.2 µM dNTP, 0.6 µM of each primer, 1 U *Taq*, and 1.0 µL DNA template (approximately 20 ng/µL).

Identification of –521 C/T genotypes was based on the restriction fragment length polymorphism (RFLP) technique. PCR products were digested using *Fsp*I (recognition sequence: 5' TGC▲GCA 3') restriction endonuclease (New England Biolabs, Beverly, MA, USA) in order to identify the alleles at -521. The restriction enzyme acts as a diagnostic tool, *Fsp*I cuts if a 'T' allele is present, and does not cut the site if 'C' allele is present. The result is two products that differ in length (176 base pairs (bp) for T, 285 base pairs for C), see Figure 5. *Fsp*I (50 U/mL) was mixed with 8 µL of PCR products, 2 µL NE Buffer 4 (see recipe in Appendix G) and water to a total volume of 20 µL which was incubated at 37°C for at least 5 hours and as long as 16 hours.

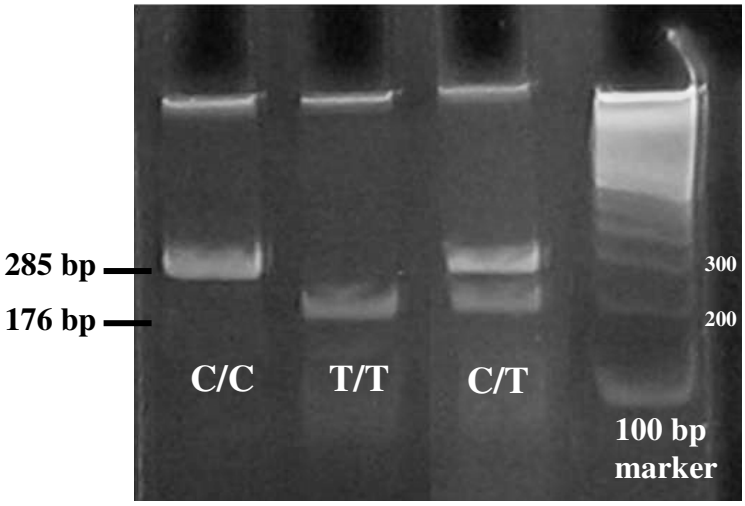


Figure 4: The -521 C/T genotypes shown in a UV-light photograph.
A single band just below the 300 bp marker represents an un-cut C/C genotype; a single band below the 200 bp marker represents two cleaved strands of DNA, or a T/T homozygote; and finally two lines denote a heterozygote genotype, C/T, with one strand that has been cleaved.

Gel electrophoresis

PCR products were separated by polyacrylamide gel electrophoresis (PAGE). PAGE gels that were 8% Acrylamide/Bis (see Appendix G for recipe) were inserted in a BioRad vertical gel electrophoresis chamber and the chamber was filled with tris-borate EDTA buffer (1 x TBE, see Appendix G). The standard running time for the gels was 45 minutes at 120 volts. PCR (8 μL) or digestion products (20 μL) were loaded into individual wells using approximately 1 μL Bromphenol Blue loading buffer (30% glycerol, 10% TE). After electrophoresis, the gels were soaked in a dilute solution of ethidium bromide (~0.5 μg/mL) to stain the PCR products. The DNA bands were visualized using ultra-violet light and the gels were digitally photographed with a Cannon Power Shot A620 with Canon LA-DC58F Lens adapter. The size of the DNA bands were determined based on their location in the gel compared to a standard 100 bp reference ladder (Invitrogen, California). The size of the uncut band amplified using F_3/R_3 primers was 285 bp (see Figure 5).

Genotyping by pyrosequencing

In a final attempt to optimize the amplification of the GC-rich region that surrounds -521 C/T, the pyrosequencing technique was employed. Primers were chosen using the Biotage AB PSQ Assay Design Software (Version 1.0.6, USA): forward primer, F1 pyro, 5' TAG GCG TCG GCG GTT GAG 3'; reverse primer, R1 pyro, 5' GAC TCG CCT CGA CCT CGT G 3'; and sequencing primer, S1 pyro 5' TCG GGG GCA GGG GGA 3'. The amplified region of the DRD4 gene (amplicon) was 82 base pairs in length. Primers were HPLC purified and the reverse primer was biotinylated at the 5' end (NAPS IDT, UBC, Vancouver).

Sequencing for pyrosequencing

Preliminary pyrosequencing data reported sequences that did not match sequence data (*i.e.* reporting numerous SNPs in the region) reported in the University of California Santa Cruz (UCSC) Genome Bioinformatics database for the upstream *DRD4* region. In order to minimize pyrosequencing errors, PCR products were sent away for sequencing. Three DNA templates that had been identified by RFLP as C/C, C/T and TT respectively

were amplified with the pyro-primers. The PCR products were purified using Qiagen QIAquik PCR Purification Kit (Qiagen Sciences, Maryland) and analyzed in the CMMT/BCRI DNA Sequencing Core Facility (UBC, Vancouver).

PCR for pyrosequencing

DNA was amplified using the following PCR conditions: 95°C for 5 minutes, followed by 49 cycles of 95°C for 20s, 60°C for 20s and 72°C for 20s, followed by a chase of 5 minutes at 72°C. The 15 µL reactions contained 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.5 mM $MgCl_2$, 0.2 µM dNTP, 0.4 µM of each primer, 1 U native *Taq* polymerase (Invitrogen, California), and 1.0 µL DNA (approximately 20 ng/µL).

Pyrosequencing reactions

Single-stranded biotinylated PCR products were prepared for sequencing using the Pyrosequencing Vacuum Prep Tool (Biotage AB, Uppsala, Sweden) (Zhou et al., 2006). Two microlitres of streptavidin-coated sepharose beads (Biotage AB) was added to 38 µL of binding buffer (Biotage AB) and 25 µL of distilled water and was then mixed with 15 µL of PCR product for approximately 20 minutes using a Labnet Orbit P2 Shaker. The beads bound to the PCR products which then adhered to the vacuum filter probes. The vacuum was washed for 5 s each in 70% ethanol, followed by a denaturation solution (0.2 M NaOH), and finally a washing buffer (10 mM Tris-acetate). The vacuum was then disabled, and the beads were released from the individual probes into a PSQ HS 96 Plate (Biotage AB) containing a solution of 0.4 µM sequencing primer (S1 pyro) and 19.2 µL annealing buffer (Biotage AB) per 20 µL reaction.

The PSQ 96 SNP Reagent Kit (Biotage AB) containing 182 µL enzyme, 182 µL substrate and a solution of each nucleotide (volumes based on the software instructions once the amplicon sequence data was entered into the PyroQ SNP computer program) were dispensed into the PSQ 96 plate. The following sequence was inputted into the PyroQ SNP software: 5' GCGGGCGNGGAGGGYG 3'. Previous data suggested the presence of a SNP six nucleotides up-stream from the –521 C/T SNP, therefore when the sequence primer reached 'N', the machine dispensed one of each A, C, T, G. At the SNP site of interest (-521) 'Y', a C and a T was dispensed. The PyroMark MD dispensed the nucleotides in the following order, beginning the sequence with a random base (T),

TGCGCGACTGAGATC. Sample genotypes were determined using SNP Software (Biotage AB).

STATISTICAL ANALYSIS

Phase I: Exploratory factor analysis and validation of CSSQ-S

An exploratory factor analysis of the CSSQ-S was performed using SPSS Graduate Student Version 16.0 to determine whether all of the items (1 through 13) measured the same facet of personality (contextual sensation-seeking for skiing), and to determine the amount of variance accounted for by the factor(s). I chose an exploratory factor analysis (instead of a confirmatory FA) because it is a tool for generating hypotheses about relationships in the data set, and is often used in the early stages of research (Tabachnick and Fidell, 1983). The sample size for the proposed study was smaller than ideal for factor analysis, but over 200 samples is considered a "fair" sample size (Tabachnick and Fidell, 1996). Questions showing up as outliers after factor analysis were discarded from further analyses. Internal consistency measures, Cronbach alphas, were calculated to provide one measure of reliability.

Behaviour and personality

The second portion of Phase I involved comparing the CSSQ-S results against the ZKPQ ImpSS scores. Pearson's Correlation (r) was calculated to measure the association (if one exists) between global sensation seeking and contextual sensation-seeking behaviours.

Phase II: Personality and -521 C/T polymorphism

The personality variables (CSSQ-S and ZKPQ ImpSS) have continuous outcomes, therefore the data was analyzed using a blocked one-way analysis of variance (ANOVA) (based on recommendations by Balding, 2006). Sex was a blocking variable since studies have suggested that the genetic influence on traits related to sensation seeking, such as novelty seeking and extraversion, is stronger in females (Bookman et al., 2002; Ronai et al., 2002; Ono et al., 1997). Using the ANOVA method decreased the

number of tests of significance, measuring subscales and genotype frequencies simultaneously, thereby eliminating the need for multiple testing corrections.

RESULTS

Subjects

A total of 201 subjects were included in the study. Table 4 lists the descriptive statistics for the variables of interest.

Table 4: A summary of participant statistics.

	N_{Tot}	Genotype	N_{gene}	Total ImpSS	N_{SS}	CSSQ	N_{CSSQ}
Males	101	CC	14	12.84 ± 3.30	98	3.83 ± .56	100
		CT	14				
		TT	10				
Females	100	CC	15	10.80 ± 4.66	97	3.05 ± .70	100
		CT	14				
		TT	7				

Note: CSSQ scores are post-factor analysis (10-item score). The number of participants (N) is included for each stage of the analysis.

Phase I: Factor analysis and validation of CSSQ-S

The CSSQ-S[4] was examined for accuracy of data entry, missing values and outliers before employing the factor analysis. One case (033) was excluded from the factor analysis (FA) because 10 out of 13 scores were missing. Two other cases (236 and 193) had two and three missing data points respectively, which appeared to be random. The missing data points were replaced with the average value based on the other 10 or 11 responses on the questionnaire. This method is considered a conservative approach to dealing with missing data since the mean for the distribution remains the same (Tabachnick and Fidell, 1983).

Based on Mahalanobis distance ($p < .001$), no cases were identified as multivariate outliers for the CSSQ-S in combination with the ImpSS ZKPQ subscale (Tabachnick and Fidell, 1983). Linearity was confirmed by examining a simple bivariate scatterplot between the total CSSQ-S score and the ImpSS subscale from the ZKPQ (Appendix I).

Finally, the CSSQ-S continuous variables were screened for normality. Both skewness and kurtosis of each variable was examined ($z_{critical} = \pm 2.58$, $p < .01$) to

[4] Recall: CSSQ-S is the contextual sensation-seeking questionnaire developed for a ski population.

determine the normality of the data set. This procedure indicated that the CSSQ-S scores were normally distributed (See Appendix J).

Exploratory factor analysis (FA) of CSSQ-S

Maximum likelihood (ML) extraction and varimax rotation were performed on the 13-item CSSQ-S using SPSS 16.0 statistical software. The items had been previously screened for missing data, outliers, linearity and normality. Factor analysis is a statistical method often used to determine the number of factors assessed by a questionnaire, in this case the newly developed CSSQ-S. A varimax rotation was chosen because it maximizes the variance of the items within each factor (Tabachnick and Fidell, 1983).

The minimum criterion for a factor to be meaningful was set at an eigenvalue[5] greater than 1, which is convention (Tabachnick and Fidell, 1983). Each factor loading represents the amount of variance shared with the overall factor, a criteria of .30 represents a 9% overlap of variance and is often used as the inclusion minimum (Tabachnick and Fidell, 1983), however since the study sample was smaller than ideal, a more conservative cut-off value of .40 (representing a 15% overlap) was used for factor loadings.

Two factors with eigenvalues greater than 1 were extracted from the exploratory FA, $\lambda_1 = 4.38$ and $\lambda_2 = 1.78$, accounting for 33.67% and 13.67% respectively of the total explained variance. The scree test was also used to provide a visual estimate of the number of factors (Appendix K). Loadings of variables on factors 1 and 2, communalities and percents of variance are shown in Appendix L. Two items loaded strongly with Factor 2, which will be named 'etiquette' (.666 and .984), while the other 10 items loaded onto Factor 1 'contextual sensation seeking' (factor loading range from .530 to .731) (Appendix L). Factor 1 accounted for a greater proportion of the total variance compared to Factor 2. One item (Q13, "If I see a "danger of avalanche" sign I will usually try to find another safer route") was omitted from analysis because its loading after rotation was less than .40. The two items that were selected for deletion due to high loadings on the factor 2 (etiquette) were Q11 and Q12 on the questionnaire ("I

[5] Eigenvalues are algebraic transformation variables that represent the variance accounted for by each factor (for more information, see Tabachnik and Fidell, 1983).

slow down on busy runs", and "I don't slow down on busy runs, instead I just dodge people").

The final questionnaire consisted of 10 items measuring a single factor, namely 'contextual sensation seeking'. Factor analysis of the 10-item questionnaire was employed to confirm that the questionnaire measured a single factor (contextual sensation seeking) and to determine the amount of variance accounted for by the factor. The factor matrix for the 10-item questionnaire is shown below in Table 5. Variables are ordered by the size of loading to facilitate interpretation and loadings under .4 were omitted. One eigenvalue, 5.010 accounted for 50.096% of the variance (Scree plot shown in Appendix M). The factor loadings for the 10 items in the questionnaire ranged from .539 to .786.

Table 5: Factor analysis of 10-item CSSQ-S: factor loadings, alpha, variances.

CSSQ-S Item	Factor 1*	Communalities
Q6 Push limits	.786	.618
Q4 Out of bounds	.750	.562
Q9 Novelty	.745	.555
Q1 Speed	.707	.499
Q10 Cliff jump	.689	.475
Q8 Straight line	.651	.424
Q2 Explore	.630	.397
Q7 Lose control	.577	.333
Q3 Unknown	.562	.316
Q5 Quality of jump	.539	.290
Eigenvalue	5.010	
% variance	50.096	

*Factor 1 represents contextual sensation seeking. Factor loadings are shown after three items (Q11, 12, 13) were removed from the questionnaire.

Internal consistency

The Cronbach alpha was used to measure the internal consistency of the questionnaires. The 10-item CSSQ-S had an alpha of .882. This reflects a very high internal consistency (Clark and Watson, 1995) indicating that each item in the scale measured the same facet of personality (contextual sensation-seeking in skiing and snowboarding). The mean inter-item correlation was .442 (range .279 to .641) and the mean inter-item covariance was .491 (.218 to 1.02). Overall item mean was 3.44 out of a maximum score of 5 (individual item means listed in Appendix J).

Consensual validity check

Out of the 201 samples collected, a peer review supplemented 62 samples. The responses to all of the items on the CSSQ-S peer version were compared against the participant CSSQ-S using a one-way ANOVA. There was no significant difference between the peer versions and the participant CSSQ ($p>.05$) (Appendix N).

Correlation of CSSQ-S and ZKPQ

The two measures of sensation seeking were screened for outliers, normality and linearity (See Appendix I, J, and P). Both the CSSQ and the ZKPQ satisfied all of the assumptions required for a correlation analysis. Pearson's correlation coefficient between the two sensation seeking scales was calculated using SPSS and revealed a significant association between the measures of global (ZKPQ ImpSS) and context-specific (CSSQ-S) sensation seeking ($r^2 = .506$, $p < .001$) (Appendix O).

Phase II: Personality and the -521 C/T polymorphism

Personality questionnaire: ZKPQ data

The ZKPQ was examined for accuracy of data entry, missing values and outliers using SPSS before the genetic association analysis was carried out. The missing data from the ImpSS subscale was substituted with mean values, however a modified approach was required since the responses were dichotomous and the total score was a tally of points rather than an average. Specifically, the total ImpSS score was divided by the maximum score for the individual and this value was inserted in place of the missing value. The data were also screened for outliers using Mahalanobis distance detailed above (prior to FA of CSSQ-S). The assumptions of linearity and normality were met (Appendix P).

The infrequency scale is the sixth subscale of the ZKPQ. It, in itself is not a separate factor, but a way of eliminating subjects. The items comprising the infrequency scale are exaggerated or socially desirable, but unlikely to be true for anyone. In Zuckerman's questionnaire validation, only 4% of subjects scored higher than 3 on the scale (three true responses), and therefore this was set as the cut-off in a previous study (Zuckerman et al., 1993). In the current sample, 26% of subjects scored 3 or higher and

13% scored 4 or higher. This high number of infrequency scores prompted a closer look into the items. One item "No matter how hot or cold it gets, I am always quite comfortable" may be biased in this group of skiers who may have a tolerance to extreme temperatures due their choice of sport. Upon eliminating this item from the infrequency scale, the high scorers (greater than 3) dropped to 6% (or 12 subjects). Further, another question on the scale "I never get lost, even in unfamiliar places" had a high response rate, 41 individuals (20%) answered true (scoring a point on the infrequency scale). With such a high response rate, this item does not fit the criteria for an infrequency item in this particular population, thus the item was removed. After modifying the infrequency items, 3% (N = 6)[6] of the ski population scored higher than 3 and therefore were removed from the analysis.

In accordance with the hypothesis that skiers and snowboarders would exhibit higher levels of sensation seeking than the general population, the population of skiers and snowboarders displayed significantly higher impulsive sensation-seeking scores. The mean ImpSS score for skiers was 11.82 (SD = 4.15) out of a maximum of 19, which was significantly different than 'norms' described elsewhere (mean = 10.18, SD = 4.10, N = 2969) (Zuckerman et al., 1993), $t = 5.47$, $p < .01$ ($df = 3167$). Consistent with previous studies, males score significantly ($t_{obs} = 3.51$, $p<.01$) higher than females (Table 6). The distribution of scores in the ImpSS for males and females is shown in Appendix Q.

Table 6: Means and standard deviations for males and females scores on the Zuckerman Kuhlman Personality Questionnaire (ZKPQ).

	Males (N=98)		Females (N=97)	
ZKPQ	Mean	SD	Mean	SD
ImpSS	12.84	3.30	10.80	4.66
Agg-Hos	7.08	3.17	6.46	3.20
Neu-Anx	4.40	3.42	7.63	4.14
Soc	8.42	3.39	9.78	3.58
Activity	9.79	3.56	10.16	3.57

Note: The cases with high infrequency scores have been removed at this stage of the analysis.

[6] The cases that were removed from further analyses using ZKPQ were 024, 044, 106, 208, 287, and 332.

Genetic data

The concentrations of 175 DNA templates were measured using spectrophotometry because there was large variability between samples. The average concentration was 21.3 ng/μL (± 22.4 ng/μL).

Polymerase chain reaction (PCR)

Using the PCR and RFLP methods, the –521 C/T SNP genotypes of as many individuals as possible were confirmed. The DNA of all 201 subjects was isolated and PCR was attempted, however a total of only 59 subjects' genotypes were confirmed via the PCR RFLP method (a representative photograph depicting digested PCR products is shown in Appendix R). A summary table in Appendix S includes genotypes, CSSQ-S scores and ZKPQ ImpSS scores of the individuals whose DNA successfully amplified by either the pyrosequencing and/or RFLP methods (total N = 74).

DRD4 sequencing

Sequence data for the 82 bp amplicon using the pyrosequencing primers is shown in Appendix T. The sequence was consistent with some of the literature, although additional SNPs reported by the UCSC genome informatics centre did not appear in any of the samples. The ancestral alleles were therefore used for pyrosequencing (with the exception of the -521 C/T SNP and a SNP at -515).

Pyrosequencing *DRD4* -521 C/T

A total of 25 genotypes were confirmed using pyrosequencing methods. Ten of the genotypes had already been identified by RFLP, and genotypes identified by both methods were consistent. A sample pyrosequencing result is attached in Appendix U.

Hardy-Weinberg equilibrium

A Chi square test was used to compare observed allele frequencies with expected frequencies to verify that the population is in Hardy-Weinberg equilibrium (HWE), indicating the presence of random mating. A HWE test may provide additional evidence for an association if our test population is not in equilibrium. The allele frequencies for the sample of skiers and snowboarders were .58 C and .42 T. The genotype frequencies

were .39 C/C, .38 C/T and .23 T/T. The observed frequencies were compared to the expected frequencies resulting in a Chi square value of 3.67, this is slightly less than the critical value (χ^2 = 3.84, df = 1, p = .05), therefore the population did not deviate from Hardy Weinberg Equilibrium.

The allele frequencies for the ski population were not consistent with Caucasian population data (recall Swedish 0.42 C:0.58 T and Russian 0.43 C:0.57 T, Table 2). Comparing the ski population in a 2x2 contingency table with the data from Jonsson and colleagues' (2002) Swedish population data (N = 368), a significant difference in allele frequencies between the two populations was found (p < .001) (Appendix V).

One-way blocked ANOVA: ZKPQ and genetics

Using the statistical software Gpower[7], the sample size N=201 for the group was large enough to detect an effect size of .30 (based on Schinka et al., 2002) at a power of .95 with alpha set at .05 using a one-way ANOVA with two levels. Unfortunately, due to difficulties amplifying the *DRD4* -521 C/T SNP, the sample size for the genetic analysis was reduced to N = 74. This sample size had insufficient power (<.8) to detect a small effect size, nonetheless genetic data was analyzed in association with personality parameters.

The sample (N = 74) was analyzed by one-way ANOVA with sex as the blocking variable and genotype as the independent fixed factor (2 levels), against the CSSQ and ImpSS scores (dependent). The ANOVA assumptions were upheld and the personality scores were normally distributed (based on Q-Q plots) and the variances were not significantly different (Levene's test for homogeneity of variance, p > .05 for each dependent variable) (Balding, 2006). A sample of the blocks of the ANOVA is shown in Appendix W. The number of cases per block are not equal, however, because the ratio between blocks (*i.e.* female C/C genotype block N = 15, female C/T or T/T genotype block N = 21) is close to 1.5, the type of sums of squares does not need to be modified (Stevens, 1999). The default Type III sums of squares was therefore employed in the analysis. Males and females were analyzed separately because it was hypothesized that

[7] An online statistical software that calculates power (http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/)

the relationship between genotype and sensation-seeking scores would differ between males and females.

The grouping of genotypes for the analysis was based both on the literature and an independent t-test between genotype scores in females. A significant difference between the CSSQ-S means for the C/C genotype compared to the C/T group was found ($t = 3.418$, $df = 27$, $p = .002$), whereas no significant difference was found between the mean scores of the C/T and T/T genotypes ($t = 1.79$, $df = 19$, $p = .09$). The T allele was thus assigned dominance, and C/T and T/T genotypes were grouped together. The sensation-seeking scores for males and females by genotype are summarized in Table 7.

Table 7: A summary of sensation-seeking scores by genotype for males and females.

Sex	Genotype	CSSQ-S score	ZKPQ ImpSS
Males	C/C (n = 14)	3.89 (.63)	14.21(3.23)
	C/T & T/T (n = 24)	3.75 (.48)	13.17 (3.27)
Females	C/C (n = 15)	3.43 (.61)	12.73 (4.25)
	C/T & T/T (n = 21)	2.83 (.60)	10.10(4.52)

ZKPQ and CSSQ-S results are given as mean raw scores (SD).

There were no significant differences in mean sensation-seeking scores (ImpSS and CSSQ-S) between genotype groups for males ($p_{ImpSS} = .345$, $p_{CSSQ\text{-}S} = .473$) (Appendix Y). There was, however, a significant difference in sensation-seeking scores between genotypes for the females. Females with C/C genotypes had significantly higher CSSQ-S scores than females with C/T or T/T genotypes ($p = .006$) (Table 7 and Appendix X). The effect size of the relationship between genotype and CSSQ-S score in females was low to moderate ($\eta^2 = .2$). A trend between genotype and ImpSS score was also observed in females, and although it was not significant ($p = .086$), the C/C group had *higher* scores than the C/T, T/T group (Table 7 and Appendix X). Genotype had a small effect on ImpSS scale in females ($\eta^2 = .08$).

DISCUSSION

The primary purpose of this investigation was to determine whether an association exists between the alleles of the -521 C/T polymorphism of the dopamine-4 receptor and levels of sensation seeking in skiers and snowboarders. In order to measure the association between genetics and sensation seeking, the CSSQ-S was developed and validated to serve as a precise means of phenotype identification in addition to the global ZKPQ ImpSS scale. Overall, an association between the alleles of the -521 C/T polymorphism and sensation-seeking was found in females, however no association was found in males. Precise phenotype identification coupled with separate analyses by sex may have contributed to strength of the study design.

Phenotype identification

Following Eisenberg and colleagues (2007), this investigation is the second to analyze the sensation-seeking trait in association with a *DRD4* polymorphism. Previous studies have investigated novelty seeking and extraversion in relation to *DRD4* polymorphisms and have resulted in inconsistent findings (reviewed by Schinka et al., 2002 and Munafo et al., 2008). Sensation seeking is highly related to novelty seeking (Zuckerman and Cloninger, 1996; Zuckerman and Kuhlman, 2000), however novelty-seeking does not describe the quality of sensations that are sought (*e.g.* intense and complex sensations) (Zuckerman, 2005). Perhaps certain types of sensations have a greater effect on dopamine-mediated approach behaviours.

Unsuccessful replications of genetic association studies may be attributable to the failure to identify the corresponding phenotype. One great challenge of psycho-genetic studies is the identification of precise phenotypes, since inaccurate phenotype classification leads to incorrect results (Kreek et al., 2005). The questionnaire that measures novelty seeking, the TCI, has been criticized for having psychometric weakness including low internal consistencies and heterogeneous subscales (Cloninger, 1987; Reuter and Hennig, 2005; Gana and Trouillet, 2003). The psychometric weakness of the TCI may be a possible explanation for inconsistent replications of the -521 C/T associations.

In order to measure the sensation-seeking trait, I employed the ZKPQ rather than the older SSS forms because the former contained more relevant (and current[8]) questions for my study population. ZKPQ has shown high content and construct validity in previous studies, meaning that the sensation-seeking subscale measures all facets of the trait and inferences about the personality trait may be based on the scores obtained (Zuckerman et al., 1993). Furthermore, the ZKPQ is a scale that has shown high retest reliability (.82 to .87, retest interval = 2 months) (Zuckerman and Kuhlman, 2000). Retest reliability is important because failures to find associations between the DRD4 gene and personality traits may have been due to the lack of uniformity employed when measuring the personality trait (Lusher, Chandler, & Ball, 2001).

In summary, sensation-seeking was measured as opposed to novelty seeking, because the latter only describes a portion of the sensation-seeking concept (Zuckerman, 2005). In addition to the ZKPQ, a new questionnaire (the CSSQ-S) that measures specific behaviours associated with sensation seeking was developed to provide an additional means of phenotype identification.

Sensation-seeking personality and behaviours

A main objective of the study was to create a sport-specific questionnaire (the CSSQ-S) that measures sensation-seeking behaviours in skiers, and to evaluate how closely it relates to Zuckerman's impulsive sensation-seeking scale. An exploratory factor analysis of the CSSQ-S for the entire sample (N = 200) was employed and revealed a two-factor solution.

A total of three items were removed from the questionnaire based on the results of the exploratory factor analysis. One of the items was deleted because of inadequate loading (< .4), and the two other items loaded separately onto a second factor that was named "etiquette". The two items from factor 2 selected for deletion were highly related (inter-item correlation = .71) and stated "I slow down on busy runs" and "I don't slow down on busy runs, instead I just dodge people". Conceptually, the items do not represent the sensation-seeking definition, and appear to be a measure of "etiquette" or

[8] An example of an out-dated item, "I would like to meet some persons who are homosexuals" and an irrelevant item, "I like some of the earthy body smells".

"courtesy" instead. The item that failed to load onto either factors stated, "If I see a 'danger of avalanche' sign I will usually try to find another safer route", which is not applicable to subjects who have never been in avalanche terrain, and therefore was not an appropriate question for this sample population.

A second factor analysis was employed to confirm the factor structure. The factor analysis of the 10-item CSSQ-S revealed that all items loaded onto a single factor, that of "contextual sensation seeking in skiing". Each item appeared to represent a different facet of the factor since the factor loadings were neither too high nor too low (.539 to .786). In scale development it is recommended that developers strive for internal consistencies (measured by Cronbach α) of at least .80 (Clark and Watson, 1995). The internal consistency of the CSSQ-S surpassed this criterion (α = .88). A high internal consistency reveals that the questionnaire is measuring what it is supposed to measure, responders were consistent between items.

High scorers on the ZKPQ ImpSS scale in general exhibit greater sensation-seeking behaviours in the context of skiing (based on the CSSQ-S). Global sensation seeking and contextual sensation seeking are related (r^2 = .506), which confirms the hypothesis of a moderate correlation between the two measures. Conceptually, it makes sense that one would behave on the slopes similarly to how they would behave in life. There are some exceptions, however, that may influence behaviour, one such being ability. For example, an intermediate skier may sky-dive and act without inhibitions in day-to-day life, but lacks the ability to display the risk-taking, sensation-seeking behaviours in the context of skiing. For this reason, the current study excluded beginner skiers which may have contributed to the strong relationship observed between ski behaviours and personality.

Many studies have compared global sensation-seeking scores between high- and low-risk activities; however few studies have investigated the relationship between the sensation-seeking personality trait and context-specific high-risk behaviours. More importantly, the creation of a situation-specific questionnaire (CSSQ-S) that measures behaviours associated with sensation seeking provides more precise phenotype identification for this candidate gene association study.

DRD4 gene association

The higher sensation-seeking scores in skiers compared with other populations supports previous associations that high-sensation seekers often involve themselves in high-risk activities (Bouter et al., 1988; Diehm and Armatas, 2004; Franken et al., 2006; Zuckerman, 2000). The hypothesis that an over-representation of the C allele would be present in this study population, given that skiing is often considered a high-risk sport, was confirmed. Compared with a Caucasian (Swedish) college population, the sample of skiers had a significantly higher frequency of -521 C alleles ($\chi^2 = 11.8$, $p < .01$). The skiers were a self-selected (non-random) population, and therefore a deviation from Hardy Weinberg Equilibrium was expected, however possibly due to the small sample size (N = 74) the deviation was not significant ($\chi^2_{observed} = 3.67$, $\chi^2_{critical} = 3.84$).

Previously, the grouping of genotypes (*i.e.* assigning a dominant allele) has varied between studies. In the current study, the T allele was assigned dominance based on the observation of a smaller difference in scores between the C/T and T/T genotypes. The C/T and T/T genotypes were therefore combined into a single group. In support of the interaction hypothesis, a stronger association between the alleles of the -521 C/T SNP and sensation-seeking behaviours (CCSQ-S score) in skiing ($p = .006$), along with a similar trend in sensation-seeking personality scores ($p = .086$), was found in females. Females having the C/C genotype displayed the highest sensation-seeking ski-behaviours compared to the C/T and T/T genotypes. The presence of the C allele (in a homozygote) may have a stronger influence on the sensation-seeking levels of females over males. It was unanticipated that no association (nor trend) was observed in the male study population ($p_{CSSQ} = .345$, $p_{ImpSS} = .473$). These results prompt an interesting question: what are possible causes of these sex differences?

Sex differences

Inconsistent and non-significant findings in previous *DRD4* association studies may be due to the fact that a small number of studies included only males (Ebstein et al., 1996; Okuyama et al., 2000; Benjamin et al., 1996). Previous studies that have combined the sexes for analysis may have missed the relationship between genetics and the novelty/sensation-seeking trait in females, which may have a stronger genetic basis than

in males (Stoel et al., 2006). Furthermore, previous studies that have included only females, or carried out separate analyses, found associations between novelty seeking and extraversion and the alleles of the -521 C/T polymorphism (Bookman et al., 2002; Lee et al., 2003; Ronai et al., 2001). Sex may therefore be an important independent factor to consider when designing the statistical analysis of behavioural-genetic association studies.

In the current study females scored significantly lower ($M_{females}$= 10.80, t_{obs} = 3.51, $p < .01$) than males (M_{males}= 12.84) on the ImpSS scale and on the CSSQ-S scale (M_{female} = 3.05(.7), M_{males}= 3.83(.6), t_{obs} = 8.7), and this is consistent with results from other studies (Zuckerman and Kuhlman, 2000; Zuckerman et al., 1993). From an evolutionary perspective, lower levels of risky behaviours exhibited by females may be in part due to the distinct biological roles males and females play (Zuckerman, 2000). A lower score for females is not surprising given that behaviours associated with sensation seeking may threaten the survival of progeny. Inherently, females are required to invest in the long-term care of their children and therefore need to stay alive by using risk-avoidance strategies. Males, on the other hand, are only needed for conception and therefore benefit from carrying out risky behaviours that lead to increased propagation and genetic diversity through exploration (Zuckerman, 2000; Zuckerman and Kuhlman, 2000).

Although evolution and genetics play an important role in the sensation-seeking trait, the other factors that influence personality must not be overlooked and may provide insight as to why an association was found in women and not in men. Humans are social beings, and therefore the environment plays a substantial role in personality traits, possibly more so for males according to a twin study by Stoel and colleagues (2006). All of the variation in the sensation-seeking trait between female monozygotic twin-pairs and their non-twin siblings was attributable to genetics; where as a large portion of the variability in the male twin/sibling comparisons was environmental. It was suggested that varied levels of sensation seeking in males is more a product of their environment than their genetic make-up (Stoel et al., 2006). A conceivable explanation is that certain personality traits, such as impulsive sensation seeking, may be considered more socially desirable for one gender than the other. Sensation seeking has been linked to more "so-

called"[9] masculine interests and characteristics, and has been negatively associated with "so-called" feminine interests (Kish, 1971; Daitzman and Zuckerman, 1980). High levels of sensation seeking in males may be considered socially desirable because it further contributes to a stereotypical gender identity of masculinity.

Today, activities that showcase individuals with the high thrill-seeking persona (*e.g.* extreme sports) receive positive coverage in the media. Extreme sports are no longer activities for members on the fringe of society; in fact, these sports have become somewhat main-stream (Creyer et al., 2003). High-risk sports have gained a certain prestige due to increased media exposure; which may lead to increased social pressures, particularly for males, to exhibit higher levels of the sensation-seeking trait. For example, a low sensation-seeking, young man might try to flaunt sensation-seeking characteristics in order to identify with a peer group. The same pressure does not exist for women since sensation seeking is not iconic of femininity (Saxvik and Joiremen, 2005). The non-significant association between the DRD4-gene and sensation seeking levels in males may be in part due to differential social pressures faced by men and women.

LIMITATIONS

Power

Genetic association studies are often plagued by low statistical power because the effect sizes of the SNPs are often very small (Ebstein, 2006; Munafo et al., 2008). Numerous studies suggest that replications of the DRD4-gene and novelty-seeking associations have been problematic due to inadequate sample size leading to Type II error (Golimbet et al., 2005; Lee et al., 2003). On the other hand, some studies that claim adequate power failed to find associations (Jonsson et al., 2002), suggesting that previous associations may have been found by chance (Type I error).

The current study was under-powered due to difficulties encountered in genotyping the -521 C/T SNP. Power analysis suggests a minimum sample size of 138

[9] Note: I have used the term "so-called" because today the lines between gender categories are not clear-cut.

and we were only able to include a random sample of 74 individuals. Attempts to analyze the entire sample (N = 201) are ongoing.

Generalizability

This was the first study to address the genetics of sensation seeking in the field of sports. It was therefore important to maintain a homogenous sample by comparing individuals within one sport group rather than across different sports (*e.g.* mountain bikers versus golfers) at the cost of lower generalizability. Skiing and snowboarding are classified as high-risk sports, but it was hypothesized that these particular sports would include a number of high-sensation seekers, while still including a number of lower sensation seekers. Because the sample criteria included skiers of at least an intermediate level the distribution of ImpSS scores was expected to be higher than the general population. This sample of skiers and snowboarders scored on average 1.5 points higher on the ImpSS scale when compared with sample means from a general population of college students (Zuckerman et al., 1993). The advancements in ski ability required to reach an intermediate level involve a shift towards more challenging terrain (along with the speed and exposure that accompanies). This may explain the presence of higher ImpSS scores in the present study, as the low-sensation seekers may be weeded out of the sport at the novice level.

Potential confounding variables

Participants' familial situations may be a potential confounding factor. The participant demographic section should have included a question regarding dependents. It was observed that one subject had outlying scores (both ImpSS and CSSQ-S) for her genotype group (2 SD below the mean) and under occupation, she had listed that she was a mother. Although genetic composition remains constant throughout one's life, having children may influence personality traits (*i.e.* levels of sensation seeking may decrease). A shift to lower sensation-seeking behaviours may occur in an effort to avoid risk and protect one's child.

Another confounding variable that we attempted to control was age. Exclusions for age (between 19 and 40 years) were imposed because levels of sensation seeking decrease after the age of 40 years (Zuckerman, 1979, p. 125). It has been hypothesized

that this decrease may be due to an increase in levels of an enzyme that breaks down dopamine, MAOB (Zuckerman, 1979, p. 376). Although the genetic composition of a person does not change with age, lower levels of dopamine may have an effect on sensation-seeking behaviours, thus confounding the study. A younger population (*i.e.* elementary school-aged children) would be an ideal population to study in order to avoid biological and social confounding variables.

FUTURE DIRECTIONS

A weakness of association studies is that they do not provide causal evidence; therefore studies must be replicated in different populations to ensure the findings are not due to chance alone. Further studies are needed to evaluate the contribution of the DRD4 gene to the sensation-seeking trait. A possible design may involve comparing high-risk and low-risk sport groups. For example a sample of high-risk BASE (Building, Antenna, Span (a bridge or arch) Earth (usually a cliff)) jumpers may be compared with a low-risk group (golfers) to see whether an over-expression (measured using a two-by-two contingency table) of the 'C' allele exists in the BASE-jumping group compared to the golfers. Inclusion of a heterozygous population would increase the generalizability of the results.

This study included only the –521 C/T SNP because a majority of studies reported significant linkage disequilibrium between –521 C/T and the VNTR (Ekelund et al., 1999; Strobel et al., 2002). Numerous studies have failed to find an association between novelty seeking and the *DRD4* VNTR, and many concluded that the initial associations might have occurred because the repeated variant exists in linkage with another functional variant (Noble et al., 1998). In future studies, however, other polymorphisms in the DRD4-gene and in other neurotransmitter genes (there are many (Benjamin, Osher, Kotler, Gritsenko, Nemanoc, Belmaker, & Ebstein, 2000; Oak et al., 2000; Okuyama et al., 2000)) should be considered for investigation.

Other genes may also be investigated, including variations in the stathmin gene and the gene encoding MAOB. Stathmin, a gene found in high density in the amygdala (part of the limbic system), has been linked to fearlessness in mice (Shumyatsky, Malleret, Shin, Takizawa, Tully, Tsvetkov, Zakharenko, Joseph, Vronskaya, & Yin,

2005) and might be another possible candidate for sensation-seeking studies. Based on HapMap, there are few variations within and around both the stathmin gene and MAOB in the population. Many of the variations are dependent, meaning that only a few variations would need to be analyzed to see if associations with the sensation-seeking trait exist.

CONCLUSIONS

Sensation seeking is a complex personality trait that is likely influenced by a combination of genetics and environment. Variations in numerous genes, each contributing a small effect, combined with phenotypes measured by self-report are common obstacles faced by behavioural geneticists. Precise phenotype identification is essential, and at the cost of losing validity from the use of smaller sample sizes, there is a gain of generating less false positives or negatives (Kreek et al., 2005). The CSSQ-S may have provided a more precise tool for phenotype identification in females. The *a priori* decision to analyze females separately from males distinguished this study from previous ones and added to its strength. Males and females experience different social and environmental influences and should therefore be studied separately regarding genetic contributions to personality.

REFERENCES

Asghari, V., Schoots, O., van Kats, S., Ohara, K., Jovanovic, V., Guan, H.C., Bunzow, J.R., Petronis, A., & Van Tol, H.H. (1994). Dopamine D4 receptor repeat: analysis of different native and mutant forms of the human and rat genes. Molecular pharmacology 46, 364-373.

Balding, D.J. (2006). A tutorial on statistical methods for population association studies. Nature reviews 7, 781-791.

Benjamin, J., Li, L., Patterson, C., Greenberg, B.D., Murphy, D.L., & Hamer, D.H. (1996). Population and familial association between the D4 dopamine receptor gene and measures of Novelty Seeking. Nature genetics 12, 81-84.

Benjamin, J., Osher, Y., Kotler, M., Gritsenko, I., Nemanov, L., Belmaker, R.H., & Ebstein, R.P. Association between tridimensional personality questionnaire (TPQ) traits and three functional polymorphisms; dopamine receptor D4 (DRD4), serotonin transporter promoter region (5-HTTLPR) and catechol O-methyltransferase (COMT). Molecular psychiatry 5(1), 96-100.

Bookman, E.B., Taylor, R.E., Adams-Campbell, L., & Kittles, R.A. (2002). DRD4 promoter SNPs and gender effects on Extraversion in African Americans. Molecular psychiatry 7, 786-789.

Bouchard, C., Blair, S.N., & Haskell, W.L. (2007). Genetic Differences and P.A. and Health. In Physical Activity and Health (Windsor, Human Kinetics).

Bouter, L.M., Knipschild, P.G., Feij, J.A., & Volovics, A. (1988). Sensation Seeking and Injury Risk in Downhill Skiing. Personality & Individual Differences 9, 667-673.

Clark, L.A., & Watson, D. (1995). Constructing Validity: Basic Issues in Objective Scale Development. Psychological Assessment 7, 309-319.

Cloninger, C.R. (1987). A systematic method for clinical description and classification of personality variants. A proposal. Archives of general psychiatry 44, 573-588.

Collins, F.S., & Mansoura, M.K. (2001). The Human Genome Project. Revealing the shared inheritance of all humankind. Cancer 91, 221-225.

Consortium (2004). Finishing the euchromatic sequence of the human genome. Nature 431, 931-945.

Cordell, H.J., & Clayton, D.G. (2005). Genetic association studies. Lancet 366, 1121-1131.

Creyer, E.H., Ross Jr, W.T., & Evers, D. (2003). Risky recreation: an exploration of factors influencing the likelihood of participation and the effects of experience. Leisure Studies, 239.

Daitzman, R., & Zuckerman, M. (1980). Disinhibitory sensation seeking, personality and gonadal hormones. Personality & Individual Differences 1, 103-110.

Diehm, R., & Armatas, C. (2004). Surfing: an avenue for socially acceptable risk-taking, satisfying needs for sensation seeking and experience seeking. Personality and Individual Differences 36, 663-677.

Ebstein, R.P. (2006). The molecular genetic architecture of human personality: beyond self-report questionnaires. Molecular psychiatry 11, 427-445.

Ebstein, R.P. (2000). Association between tridimensional personality questionnaire (TPQ) traits and three functional polymorphisms: dopamine receptor D4 (DRD4), serotonin transporter promoter region (5-HTTLPR) and catechol O-methyltransferase (COMT). Molecular psychiatry 5, 96-100.

Ebstein, R.P., Novick, O., Umansky, R., Priel, B., Osher, Y., Blaine, D., Bennett, E.R., Nemanov, L., Katz, M., & Belmaker, R.H. (1996). Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of Novelty Seeking. Nature genetics 12, 78-80.

Eichhammer, P., Sand, P.G., Stoertebecker, P., Langguth, B., Zowe, M., & Hajak, G. (2005). Variation at the DRD4 promoter modulates extraversion in Caucasians. Molecular psychiatry 10, 520-522.

Eisenberg, D.T., Mackillop, J., Modi, M., Beauchemin, J., Dang, D., Lisman, S.A., Lum, J.K., & Wilson, D.S. (2007). Examining impulsivity as an endophenotype using a behavioral approach: a DRD2 TaqI A and DRD4 48-bp VNTR association study. Behavioral Brain Function 3, 2.

Ekelund, J., Lichtermann, D., Jarvelin, M.R., & Peltonen, L. (1999). Association between novelty seeking and the type 4 dopamine receptor gene in a large Finnish cohort sample. The American journal of psychiatry 156, 1453-1455.

Estabrooks, P.A., & Carron, A.V. (2000). The physical activity environment questionnaire: An instrument for the assessment of cohesion in exercise classes for older adults. Group Dynamics 4, 230-243.

Franken, I.H., Zijlstra, C., & Muris, P. (2006). Are nonpharmacological induced rewards related to anhedonia? A study among skydivers. Progress in neuro-psychopharmacology & biological psychiatry 30, 297-300.

Franques, P., Auriacombe, M., Piquemal, E., Verger, M., Brisseau-Gimenez, S., Grabot, D., & Tignol, J. (2003). Sensation seeking as a common factor in opioid dependent subjects and high risk sport practicing subjects. A cross sectional study. Drug and alcohol dependence 69, 121-126.

Gana, K., & Trouillet, R. (2003). Structure invariance of the Temperament and Character Inventory (TCI). Personality and individual differences 35.

Gelernter, J., Kranzler, H., Coccaro, E., Siever, L., New, A., & Mulgrew, C.L. (1997). D4 dopamine-receptor (DRD4) alleles and novelty seeking in substance-dependent, personality-disorder, and control subjects. American journal of human genetics 61, 1144-1152.

Golimbet, V.E., Gritsenko, I.K., Alfimova, M.V., & Ebstein, R.P. (2005). Polymorphic markers of the dopamine D4 receptor gene promoter region and personality traits in mentally healthy individuals from the Russian population. Human genetics 41, 789-793.

Goma-i-Freixanet, M., Wismeijer, A.A., & Valero, S. (2005). Consensual validity parameters of the Zuckerman-Kuhlman personality questionnaire: evidence from self-reports and spouse reports. Journal of personality assessment 84, 279-286.

Hur, Y.M., & Bouchard, T.J., Jr. (1997). The genetic correlation between impulsivity and sensation seeking traits. Behavioral genetics 27, 455-463.

Jack, S.J., & Ronan, K.R. (1998). Sensation seeking among high- and low-risk sports participants. Personality & individual differences, 1063.

Jonsson, E.G., Ivo, R., Gustavsson, J.P., Geijer, T., Forslund, K., Mattila-Evenden, M., Rylander, G., Cichon, S., Propping, P., Bergman, H., et al. (2002). No association between dopamine D4 receptor gene variants and novelty seeking. Molecular psychiatry 7, 18-20.

Kajtna, T., Tu, Scaronak, M., Bari, Cacute, R., & Burnik, S. (2004). Personality in High-Risk Sports Athletes. Kinesiology 36(1), 24-34.

Kelly, T.H., Robbins, G., Martin, C.A., Fillmore, M.T., Lane, S.D., Harrington, N.G., & Rush, C.R. (2006). Individual differences in drug abuse vulnerability: d-amphetamine and sensation-seeking status. Psychopharmacology 189, 17-25.

Keltikangas-Jarvinen, L., Raikkonen, K., Ekelund, J., & Peltonen, L. (2004). Nature and nurture in novelty seeking. Molecular psychiatry 9, 308-311.

Kereszturi, E., Kiraly, O., Barta, C., Molnar, N., Sasvari-Szekely, M., & Csapo, Z. (2006). No direct effect of the -521 C/T polymorphism in the human dopamine D4 receptor gene promoter on transcriptional activity. BMC molecular biology 7.

Kish, G.B. (1971). CPI correlates of stimulus-seeking in male alcoholics. Journal of clinical psychology 27, 251-253.

Kluger, A.N., Siegfried, Z., & Ebstein, R.P. (2002). A meta-analysis of the association between DRD4 polymorphism and novelty seeking. Molecular psychiatry 7, 712-717.

Koopmans, J.R., Boomsma, D.I., Heath, A.C., & van Doornen, L.J. (1995). A multivariate genetic analysis of sensation seeking. Behavioral genetics 25, 349-356.

Kreek, M.J., Nielsen, D.A., Butelman, E.R., & LaForge, K.S. (2005). Genetic influences on impulsivity, risk taking, stress responsivity and vulnerability to drug abuse and addiction. Nature neuroscience 8, 1450-1457.

Lee, H.J., Lee, H.S., Kim, Y.K., Kim, S.H., Kim, L., Lee, M.S., Joe, S.H., Jung, I.K., Suh, K.Y., & Kim, S. (2003). Allelic variants interaction of dopamine receptor D4 polymorphism correlate with personality traits in young Korean female population. American journal of medical genetics part B neuropsychiatric genetics 118, 76-80.

Lusher, J.M., Chandler, C., & Ball, D. (2001). Dopamine D4 receptor gene (DRD4) is associated with Novelty Seeking (NS) and substance abuse: the saga continues. Molecular psychiatry 6, 497-499.

McCourt, W.F., Gurrera, R.J., & Cutter, H.S. (1993). Sensation seeking and novelty seeking. Are they the same? The Journal of nervous and mental disease 181, 309-312.

Mitsuyasu, H., Hirata, N., Sakai, Y., Shibata, H., Takeda, Y., Ninomiya, H., Kawasaki, H., Tashiro, N., & Fukumaki, Y. (2001). Association analysis of polymorphisms in the upstream region of the human dopamine D4 receptor gene (DRD4) with schizophrenia and personality traits. Journal of human genetics 46, 26-31.

Morrongiello, B.A., & Lasenby, J. (2006). Finding the daredevils: development of a Sensation Seeking Scale for children that is relevant to physical risk taking. Accident; analysis and prevention 38, 1101-1106.

Munafo, M.R., Yalcin, B., Willis-Owen, S.A., & Flint, J. (2008). Association of the Dopamine D4 Receptor (DRD4) GEne and Approach-Related Personality Traits: Meta-Analysis and New Data. Biological Psychiatry 63, 197-206.

Noble, E.P., Ozkaragoz, T.Z., Ritchie, T.L., Zhang, X., Belin, T.R., & Sparkes, R.S. (1998). D2 and D4 dopamine receptor polymorphisms and personality. American journal of medical genetics 81, 257-267.

Oak, J.N., Oldenhof, J., & Van Tol, H.H. (2000). The dopamine D(4) receptor: one decade of research. European journal of pharmacology 405, 303-327.

Okuyama, Y., Ishiguro, H., Nankai, M., Shibuya, H., Watanabe, A., & Arinami, T. (2000). Identification of a polymorphism in the promoter region of DRD4 associated with the human novelty seeking personality trait. Molecular psychiatry 5, 64-69.

Okuyama, Y., Ishiguro, H., Toru, M., & Arinami, T. (1999). A genetic polymorphism in the promoter region of DRD4 associated with expression and schizophrenia. Biochemical and biophysical research communications 258, 292-295.

Ono, Y., Manki, H., Yoshimura, K., Muramatsu, T., Mizushima, H., Higuchi, S., Yagi, G., Kanba, S., & Asai, M. (1997). Association between dopamine D4 receptor (D4DR) exon III polymorphism and novelty seeking in Japanese subjects. American journal of medical genetics 74, 501-503.

Pain, M.T. (2005). Essay Risk taking in sport. In medicine and sport, pp. S33-S35.

Pecina, S., Cagniard, B., Berridge, K.C., Aldridge, J.W., & Zhuang, X. (2003). Hyperdopaminergic mutant mice have higher "wanting" but not "liking" for sweet rewards. Journal of neuroscience 23, 9395-9402.

Rebec, G.V., Christensen, J.R., Guerra, C., & Bardo, M.T. (1997). Regional and temporal differences in real-time dopamine efflux in the nucleus accumbens during free-choice novelty. Brain research 776, 61-67.

Reuter, M., & Hennig, J. (2005). Association of the functional catechol-O-methyltransferase VAL158MET polymorphism with the personality trait of extraversion. Neuroreport 16, 1135-1138.

Ronai, Z., Szantai, E., Szmola, R., Nemoda, Z., Szekely, A., Gervai, J., Guttman, A., & Sasvari-Szekely, M. (2004). A novel A/G SNP in the -615th position of the dopamine D4 receptor promoter region as a source of misgenotyping of the -616 C/G SNP. American journal of medical genetics part B neuropsychiatric genetics 126, 74-78.

Ronai, Z., Szekely, A., Nemoda, Z., Lakatos, K., Gervai, J., Staub, M., & Sasvari-Szekely, M. (2001). Association between Novelty Seeking and the -521 C/T polymorphism in the promoter region of the DRD4 gene. Molecular psychiatry 6, 35-38.

Saftlas, A.F., Waldschmidt, M., Logsden-Sackett, N., Triche, E., & Field, E. (2004). Optimizing buccal cell DNA yields in mothers and infants for human leukocyte antigen genotyping. American journal of epidemiology 160, 77-84.

Saxvik, S., & Joiremen, J. (2005). Sensation seeking, felt gender compatibility and psychosocial adjustment in women. Personality & individual differences 38, 1505-1515.

Schinka, J.A., Letsch, E.A., & Crawford, F.C. (2002). DRD4 and novelty seeking: results of meta-analyses. American journal of medical genetics 114, 643-648.

Schweizer, T.S. (2004). An Individual Psychology of Novelty Seeking, Creativity and Innovation. In Psychology (Erasmus University Rotterdam), pp. 288.

Seaman, M.I., Fisher, J.B., Chang, F., & Kidd, K.K. (1999). Tandem duplication polymorphism upstream of the dopamine D4 receptor gene (DRD4). American journal of medical genetics 88, 705-709.

Seeman, P., Guan, H.C., & Van Tol, H.H. (1993). Dopamine D4 receptors elevated in schizophrenia. Nature 365, 441-445.

Shumyatsky, G.P., Malleret, G., Shin, R.M., Takizawa, S., Tully, K., Tsvetkov, E., Zakharenko, S.S., Joseph, J., Vronskaya, S., Yin, D., et al. (2005). Stathmin, a gene enriched in the amygdala, controls both learned and innate fear. Cell 123, 697-709.

Stevens, J. (1999). Intermediate statistics: a modern approach. (Lawrence Erlbaum Associates).

Stoel, R.D., De Geus, E.J., & Boomsma, D.I. (2006). Genetic analysis of sensation seeking with an extended twin design. Behavioral genetics 36, 229-237.

Strange, P.G. (2000). Dopamine Receptors. Tocris reviews 15.

Strobel, A., Lesch, K.P., Hohenberger, K., Jatzke, S., Gutzeit, H.O., Anacker, K., & Brocke, B. (2002). No association between dopamine D4 receptor gene exon III and -521C/T polymorphism and novelty seeking. Molecular psychiatry 7, 537-538.

Tabachnick, B.G., & Fidell, L.S. (1983). Using Multivariate Stastitics (New York).

Tabachnick, B.G., & Fidell, L.S. (1996). Fundamental equations for factor analysis. In Using Multivariate Statistics, C. Woods, ed. (New York, Harper Collins College).

Unknown. The mental makeup of those who go big. In Elevation, pp. 31-32.

Van Tol, H.H., Wu, C.M., Guan, H.C., Ohara, K., Bunzow, J.R., Civelli, O., Kennedy, J.L., Seeman, P., Niznik, H.B., & Jovanovic, V. (1992). Multiple dopamine D4 receptor variants in the human population. Nature 358, 149-152.

Zhou, Z., Poe, A.C., Limor, J., Grady, K.K., Goldman, I., McCollum, A.M., Escalante, A.A., Bramwell, J.W. & Udhayakumar, V. (2006). Pyrosequencing, a High-Throughput Method for Detecting Single Nucleotide Polymorphisms in the Dihydrofolate Reductase and Dihydropteroate Synthetase Genes of *Plasmodium falciparum.* Journal of clinical microbiology, 44(11).

Zuckerman, M. (1979). Sensation Seeking: Beyond the Optimal Level of Arousal. New Jersey: Lawrence Erlbaum Associates.

Zuckerman, M. (2000). Are you a Risk-Taker? Psychology Today.

Zuckerman, M. (2005). Neurobiology of Exceptionality. The Neurobiology of Impulsive Sensation Seeking. US: Springer.

Zuckerman, M. (2006). Sensation seeking and risky behavior, Prevention and treatment of unhealthy risk-taking behavior. American Psychological Association.

Zuckerman, M., & Cloninger, C.R. (1996). Relationships between Cloninger's, Zuckerman's, and Eysenck's dimensions of personality. Personality & individual differences 21.

Zuckerman, M., & Kuhlman, D.M. (2000). Personality and risk-taking: common biosocial factors. Journal of personality 68, 999-1029.

Zuckerman, M.K., Kuhlman, D.M., Joireman, J. Teta, P., & Kraft, M. (1993). A comparison of three structural models for personality: the big three, the big five, and the alternative five. Journal of personality and social psychiatry 65, 757-768.

APPENDICES

APPENDIX A: Contextual Sensation Seeking Questionnaire for Skiing (CSSQ-S)

ID CODE:	**UBC Human Kinetics**

Genetics of sport-behaviours

Age: ☐ 19-24 ☐ 25-30 ☐ 31-40

Gender: ☐Male ☐Female

Occupation: ☐ Full Time ☐ Part Time

City of residence: _____

Highest level of education:
☐ High School ☐ Undergraduate degree ☐ Graduate degree (*i.e.* MBA/PHD/MD)

Race:
☐ White (European descent)
☐ First Nations descent
☐ Black (Carribean)
☐ Black (African)
☐ Black (other)
☐ Asian (Indian)
☐ Asian (Pakistani)
☐ Asian (Japanese)
☐ Asian (Chinese)
☐ Other, please specify: _____

Sport of choice: ☐ Skiing ☐ Snowboarding

Level of ability:
☐ Beginner ☐ Novice ☐ Intermediate ☐ Advanced ☐ Expert*
(*any terrain, *any condition)

Average number of runs skied in a day per difficulty grade:
●_____ ■_____ ♦_____ ♦♦_____ (estimate out of 10)

Number of days at the hill per season: ☐< 10 ☐10-25 ☐25-40 ☐>40

Do you wear a helmet? ☐ YES ☐ NO

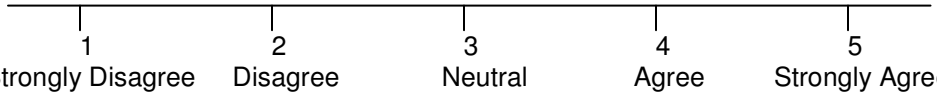
Number of ski-related injuries this season: ☐0 ☐1 ☐2 ☐> 3

Number of ski-related injuries over the last three seasons (not including this season) _____

Please complete the following questionnaire. It is a sport-specific questionnaire containing 13 specific questions about skiing/snowboarding behaviours. Please take your time to read the questions and answer truthfully. There are no right or wrong answers to any of these questions, so please just give your immediate response to the questions. You may feel that in some cases questions are repetitive but please answer every question (unless you feel uncomfortable doing so). Remember that your responses will remain confidential and no one other than the researchers involved in this study will have access to your data.

SECTION I:

Please rate the extent to which you agree or disagree with the following statements. Circle the appropriate answer.



1. I like to ski/ride fast.
   1 2 3 4 5

2. I like to ski/ride down runs that I have never been down before.
   1 2 3 4 5

3. I like to start a run even if I cannot see what lies ahead (*i.e.* big cornice).
   1 2 3 4 5

4. I like to ski/ride out of bounds.
   1 2 3 4 5

5. I like to attempt jumps even if I'm not sure of the quality of the landing area.
   1 2 3 4 5

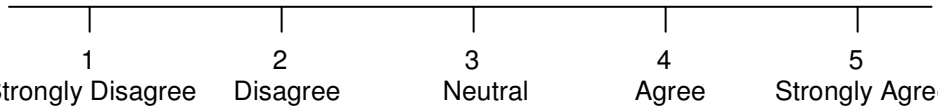
6. I like to push my boundaries when I ski/ride.
   1 2 3 4 5

7. If I lose control, I don't try to immediately slow down, I just go with it.
   1 2 3 4 5

8. If the only way down is a straight line through a narrow pass, I go for it without hesitation even if I know I will have to go fast.
   1 2 3 4 5

9. I am always trying to find new and exciting ways down a run.
   1 2 3 4 5

10. A 15-foot high drop off a cliff isn't too high a jump for me.
    1 2 3 4 5



11. I slow down on busy runs.
1 2 3 4 5

12. I don't slow down on busy runs, instead I just dodge people.
1 2 3 4 5

13. If I see a "danger of avalanche" sign, I will usually try to find another safer route.
1 2 3 4 5

THANK YOU.

WE WILL NOW PROCEED WITH THE ZKPQ STANDARDIZED PERSONALITY QUESTIONNAIRE.

APPENDIX B: Zuckerman Kuhlman Personality Questionnaire (ZKPQ)

DIRECTIONS: On the following pages you will find a series of statements that persons might use to describe themselves. Read each statement and decide whether or not it describes you. Then indicate your answer on the separate answer sheet (provided).

If you agree with the statement or decide that it describes you, answer TRUE by blacking in A on the answer sheet. If you disagree with the statement or feel that it is not descriptive of you, answer FALSE in B on the answer sheet.

A = T R U E B= F A L S E

In marking your answers on the answer sheet, be sure that the number of the statement you have just read is the same as the number on your answer sheet. Answer every statement either True (A) or False (B) even if you are not entirely sure of your answer.

1. I tend to begin a new job without much planning on how I will do it.
2. I do not worry about unimportant things.
3. I enjoy seeing someone I don't care for humiliated before other people.
4. I never met a person that I didn't like.
5. I do not like to waste time just sitting around and relaxing.
6. I usually think about what I am going to do before doing it.
7. I am not very confident about myself or my abilities.
8. When I get mad, I say ugly things.
9. I tend to start conversations at parties.
10. I have always told the truth.
11. It's natural for me to curse when I am mad.
12. I do not mind going out alone and usually prefer it to being out in a large group.
13. I lead a busier life than most people.
14. I often do things on impulse.
15. I often feel restless for no apparent reason.
16. I almost never litter the streets.
17. I would not mind being alone in a place for some days without any human contacts.
18. I like complicated jobs that require a lot of effort and concentration.
19. I very seldom spend much time on the details of planning ahead.

ZUCKERMAN-KUHLMAN PERSONALITY QUESTIONNAIRE

M. Zuckerman & D.M. Kuhlman, 1993

20. I sometimes feel edgy and tense.
21. I almost never feel like I would like to hit someone.
22. I spend as much time with my friends as I can.
23. I do not have a great deal of energy for life's more demanding tasks.
24. I like to have new and exciting experiences and sensations even if they are a little frightening.
25. My body often feels all tightened up for no apparent reason.
26. I always win at games.
27. I often find myself being “the life of the party”.
28. I like a challenging task much more than a routine one.
29. Before I begin a complicated job, I make careful plans.
30. I frequently get emotionally upset.
31. If someone offends me, I just try not to think about it.
32. I have never been bored.
33. I like to be doing things all of the time.
34. I would like to take off on a trip with no preplanned or definite routes or timetables.
35. I tend to be oversensitive and easily hurt by thoughtless remarks and actions of others.
36. In many stores you just cannot get served unless you push yourself in front of other people.
37. I do not need a large number of casual friends.
38. I can enjoy myself just lying around and not doing anything active.
39. I enjoy getting into new situations where you can't predict how things will turn out.
40. I never get lost, even in unfamiliar places.
41. I am easily frightened.
42. If people annoy me I do not hesitate to tell them so.
43. I tend to be uncomfortable at big parties.
44. I do not feel the need to be doing things all of the time.
45. I like doing things just for the thrill of it.
46. I sometimes feel panicky.
47. When I am angry with people I do not try to hide it from them.

48. At parties, I enjoy mingling with many people whether I already know them or not.
49. I would like a job that provided a maximum of leisure time.
50. I tend to change interests frequently.
51. I often think people I meet are better than I am.
52. I never get annoyed when people cut ahead of me in line.
53. I tend to start my social weekends on Thursdays.
54. I usually seem to be in a hurry.
55. I sometimes like to do things that are a little frightening.
56. Sometimes when emotionally upset, I suddenly feel as if my legs are unsteady.
57. I generally do not use strong curse words even when I am angry.
58. I would rather “hang out” with friends rather than work on something by myself.
59. When on vacation I like to engage in active sports rather than just lie around.
60. I'll try anything once.
61. I often feel unsure of myself.
62. I can easily forgive people who have insulted me or hurt my feelings.
63. I would not mind being socially isolated in some place for some period of time.
64. I like to wear myself out with hard work or exercise.
65. I would like the kind of life where one is on the move and traveling a lot, with lots of change and excitement.
66. I often worry about things that other people think are unimportant.
67. When people disagree with me I cannot help getting into an argument with them.
68. Generally, I like to be alone so I can do things I want to do without social distractions.
69. I never have any trouble understanding anything I read the first time I read it.
70. I sometimes do “crazy” things just for fun.
71. I often have trouble trying to make choices.
72. I have a very strong temper.
73. I have never lost anything.
74. I like to be active as soon as I wake up in the morning.
75. I like to explore a strange city or section of town by myself, even if it means getting lost.
76. My muscles are so tense that I feel tired much of the time.
77. I can't help being a little rude to people I do not like.

ZUCKERMAN-KUHLMAN PERSONALITY QUESTIONNAIRE

M. Zuckerman & D.M. Kuhlman, 1993

78. I am a very sociable person.
79. I prefer friends who are excitingly unpredictable.
80. I often feel like crying sometimes without a reason.
81. No matter how hot or cold it gets, I am always quite comfortable.
82. I need to feel that I am a vital part of a group.
83. I like to keep busy all the time.
84. I often get so carried away by new and exciting things and ideas that I never think of possible complications.
85. I don't let a lot of trivial things irritate me.
86. I am always patient with others even when they are irritating.
87. I usually prefer to do things alone.
88. I can enjoy routine activities that do not require much concentration or effort.
89. I am an impulsive person.
90. I often feel uncomfortable and ill at ease for no real reason.
91. I often quarrel with others.
92. I probably spend more time than I should socializing with friends.
93. It doesn't bother me if someone takes advantage of me.
94. When I do things, I do them with lots of energy.
95. I like "wild" uninhibited parties.
96. After buying something I often worry about having made the wrong choice.
97. When people shout at me, I shout back.
98. I have more friends than most people do.
99. Other people often urge me to "take it easy".

APPENDIX C: Clinical Research Ethics Board Certificate

The University of British Columbia
Office of Research Services
Clinical Research Ethics Board – Room 210, 828 West 10th Avenue, Vancouver, BC V5Z 1L8

PRINCIPAL INVESTIGATOR:	**DEPARTMENT:**	**UBC CREB NUMBER:**
James L. Rupert	UBC/Education/Human Kinetics	H07-00207

INSTITUTION(S) WHERE RESEARCH WILL BE CARRIED OUT:

Institution	Site
UBC	Vancouver (excludes UBC Hospital)

Other locations where the research will be conducted:
Whistler/Blackcomb Ski Resort Lake Louise Ski Resort Backcountry ski areas coastal and interior British Columbia Mountain Equipment Co-op

CO-INVESTIGATOR(S):
Mark R. Beauchamp
Cynthia Thomson

SPONSORING AGENCIES:
N/A

PROJECT TITLE:
The relationship between genetics and skiing/snowboarding behaviours: variants in the Dopamine receptor gene.

The current UBC CREB approval for this study expires: April 22, 2009

AMENDMENT(S):	**AMENDMENT APPROVAL DATE:**
Change in number of Subjects	**April 22, 2008**

CERTIFICATION:
In respect of clinical trials:
1. The membership of this Research Ethics Board complies with the membership requirements for Research Ethics Boards defined in Division 5 of the Food and Drug Regulations.
2. The Research Ethics Board carries out its functions in a manner consistent with Good Clinical Practices.
3. This Research Ethics Board has reviewed and approved the clinical trial protocol and informed consent form for the trial which is to be conducted by the qualified investigator named above at the specified clinical trial site. This approval and the views of this Research Ethics Board have been documented in writing.

The Chair of the UBC Clinical Research Ethics Board has reviewed the documentation for the above named project. The research study, as presented in the documentation, was found to be acceptable on ethical grounds for research involving human subjects and was approved for renewal by the UBC Clinical Research Ethics Board.

Approval of the Clinical Research Ethics Board by:

Associate Chair

APPENDIX D: Subject consent

School of Human Kinetics
210, War Memorial Gym
6081 University Boulevard
Vancouver, B.C., Canada V6T 1Z1
Tel: (604) 822-3838 Fax: (604) 822-6842

SUBJECT INFORMATION AND CONSENT FORM

Project: The relationship between genetics and skiing/snowboarding behaviours: variants in the Dopamine receptor gene.

Principal investigator: Jim Rupert, PH.D.
School of Human Kinetics
Room 346 Wesbrook Building, UBC
Vancouver, B.C., CANADA V6T 1Z2
Phone (604) 822-8462 Fax (604) 822-9451
E-mail: rupertj@interchange.ubc.ca

Other investigators:
Mark Beauchamp, PH.D.; UBC School of Human Kinetics, Annex

Cynthia Thomson, M.Sc.; UBC School of Human Kinetics

Sponsor: UBC Faculty of Education/School of Human Kinetics

Emergency Telephone Number: Dr Jim Rupert (as above) or cell (778) 386-0908

Introduction: The goals of this project are to analyze sport behaviours (sensation seeking) using a ski-specific questionnaire, and a standardized personality questionnaire (ZKPQ); and to analyze whether an association exists between common variants in the dopamine receptor gene (DRD4) and sport-specific behaviours and personality types such as sensation seeking/risk taking. The dopamine receptor gene makes the molecule that the neurotransmitter "dopamine" binds to. Neurotransmitters are the chemical signals that the brain uses to connect nerves to cells in the body.

Your participation is voluntary: Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research involves. This consent form will tell you about the study, why the research is being done, what will happen to you during the study and the possible benefits, risks and discomforts.
If you wish to participate, you will be asked to sign this form. If you do decide to take part in this study, you are still free to withdraw at any time and without giving any reasons for your decision.

Please take time to read the following information carefully. You may wish to discuss it with your family and friends before you decide. Feel free to take this form with you to read and contact the researchers at a later date if you wish to participate.

Who is conducting the study: This project is being conducted by researchers in the School of Human Kinetics at UBC. The investigators are not receiving any compensation for doing this research from any commercial organization. You are welcome to request any details concerning the funding arrangements from the Principal Investigator.

Background: A number of studies have implicated the dopamine receptor in personality and/or behavioural patterns such as sensation seeking or risk taking. Based on twin and family studies, such personality traits may be highly hereditary. Common variations in the dopamine receptor gene have been reported as over-represented in individuals displaying certain behavioural traits in a number of studies, although the evidence for a functional role for the gene is inconclusive. The genetic contribution to personality has not been studied in a sports context.

What is the purpose of the study?

Objectives: To determine whether there is a genetic association between sensation seeking/risk taking behaviours (in skiing and snowboarding), and genetic variants (alleles) in the gene encoding one of the dopamine receptors (DRD4).

The goals of the project that you are consenting to take part in are:
1) to determine the sport context behavioural patterns for each individual as measured by a ski/snowboarding behaviour questionnaire and to assess personality traits using an established personality questionnaire (ZKPQ);
2) to determine if there is an under/over representation of variants in the DRD4 among certain groups of skiers/snowboarders.

Who can participate in the study?
Men and women between 19 and 40 years of age who are at least intermediate skiers or snowboarders. The questionnaires are available in English.

Who should not participate in the study?
Given the genetic component of the study, we wish to have only one member of a family (*i.e.* only one of mother, brother, sister). Note: significant others are eligible.

What does the study involve?

The **questionnaire component** (stage 1) involves filling out a brief questionnaire on skiing/snowboarding behaviours and a standardized personality questionnaire (Zuckerman-Kuhlman Personality Questionnaire), this should take no longer than 25 minutes. You will also be given the option to have one or two friends fill out an accompanying 'peer-review' questionnaire (brief, 5 minutes), which may be mailed at their convenience, postage provided. If you feel uncomfortable answering any of the questions in the questionnaires, feel free to skip to the next one.

Buccal **DNA sample** (stage 2): A sample of cells from the inner cheek will be collected using a cytobrush (Med). This will feel similar to rubbing a firm toothbrush against your inner cheek.

Please Note: This study does not involve an actual practical ski/snowboard component.

DNA banking (optional):
As gene variants are routinely being discovered that may influence personality constructs, the investigators would like to keep the DNA samples obtained as part of this study for future genetic analysis. Declining to allow the investigators to "bank" your DNA for future, sport-behavioural studies, IN NO WAY affects your participation in "the relationship between genetics and skiing/snowboarding behaviours: variants in the Dopamine receptor gene" part of the project. If you agree to allow DNA banking, the researchers will not contact you for further consent. All guarantees of confidentially described in this consent form will apply to any future use of the DNA and you may ask to have your DNA sample destroyed at any time.

What are the possible side effects for participating in this study?
There may be a very slight tingling or irritation in the inner cheek after the buccal swabbing.

What are the benefits of participating in this study?
There are no benefits for participating in this study; however, the final results for the study will be available to you if you indicate on the consent form that you would like follow-up information.

What happens if I decide to withdraw my consent to participate?
Your participation in this research is entirely voluntary. You may withdraw from this study at any time without explanation. If you choose to enter the study and then decide to withdraw at a later time, all data collected about you during your enrolment in the study will be retained for analysis.

If you chose the option of allowing the researchers to bank your DNA samples for future, unspecified studies into the role of genetic variants in determining personality traits and sport-specific behaviours, you may withdraw from the study anytime and request that your DNA sample be destroyed.

Compensation for Injury
Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

Will my taking part in this study be kept confidential?

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. Everything (questionnaires, DNA samples, results) are coded and the key stored separately, so even in the event of a security failure in the lab or the office, it is highly unlikely that anyone could link you to the data.

Who do I contact if I want to know more about the study, or to discuss my results?
You are more than welcome to ask questions about the study at any time. Any of the investigators will be glad to discuss the results of the study with you. We welcome your comments about any aspect of the study. Please note that complex traits such as "sensation seeking" behavior are likely influenced by many genetic and environmental factors, and, while we hope the data from our study will tell us about trends in populations, the data will not be very informative at the individual level (*i.e.* we will have to look at a lot of people to see a "trend" for sensation seekers to have a specific genetic variant). The data that we will obtain from your DNA has no clinical utility or medical relevance.

Who do I contact if I have any questions or concerns about my rights as a subject during the study?
If you have any concerns about your rights as a research subject and/or your experiences while participating in this study, contact the Research Subject Information Line in the University of British Columbia Office of Research Services at 604-822-8598.

SUBJECT CONSENT TO PARTICIPATE

Project: The relationship between genetics and skiing/snowboarding behaviours: variants in the Dopamine receptor gene.

Principal investigator: Dr. Jim Rupert, School of Human Kinetics, UBC

Please initial the component(s) of the project in which you wish to participate.

I am consenting to participate in the questionnaire component of this project _____

I would like a copy of the results (provide an E-mail to which the results can be sent)_____

To participate in the DNA banking component of the study, please read the box on the next page

- I understand that by signing this form, I am consenting to participate in the study.
- I have had sufficient time to consider the information provided and to ask for advice if necessary.
- I have had the opportunity to ask questions and have had satisfactory responses to my questions.
- I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
- I understand that my participation in this study is voluntary and that I am completely free to refuse to participate, or to withdraw from this study at any time.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form.
- I have read this form and I freely consent to participate in this study.
- I have been told that I will receive a dated and signed copy of this form.
-

Printed name of subject:_____ Signature:_____Date_____

Printed name of witness:_____ Signature:_____ Date_____

Printed name of principal investigator/representative:_____ Signature:_____Date_____

Optional Voluntary Donation of Tissue for Unspecified Uses: Banking DNA samples

There are millions of common genetic variations in humans. The researchers would like to keep the DNA samples that was made from the cheek sample that you gave us for use in other experiments similar to the DRD4 experiments described in this consent form. This procedure is often called DNA banking and requires a separate consent. Please read the following, and if you are willing to allow the researchers to bank your DNA, sign below.

Storage and future use of your DNA:
Your DNA will be stored in a secure lab at UBC and will not carry any personal identifiers (*i.e.* it will be coded by a number). Any future use of the DNA will be overseen by the Principal Investigator (Jim Rupert) listed in the consent form (*i.e.* the DNA will not be provided to other researchers - if Dr. Rupert is working with other investigators, the DNA samples will remain in his lab and under his control). Before the DNA can be used for any future studies by Dr. Rupert, the proposed research will be evaluated and approved by the Clinical Research Ethics Board at UBC to confirm that the DNA is not being used for studies that differ from those to which you have consented. No attempt will be made to link your DNA to that of any member of your family. The researchers are interested only in general population categories, not in individuals, families or pedigrees.

Consent for DNA banking

I agree that the researchers may use the DNA sample obtained from me as part of this project in future similar studies of the role of genetics in behavioral traits (sensation/novelty seeking or risk taking) in sport. I understand that the investigators will not contact me to request further consent for these studies and that the DNA may be kept indefinitely (unless I request that it be destroyed). I understand that allowing my DNA sample to be banked for future studies is completely optional, and that declining to do so does not effect my participation in the other components of this study.

Printed name of subject:_____ Signature:_____ Date_____

Printed name of witness:_____Signature:_____Date_____

Printed name of principal
Investigator/representative:_____Signature:_____Date_____

You are free to withdraw, without explanation, from the DNA banking component of the project anytime you wish. To do so, simply contact Dr. Rupert (604) 822-8462 or rupertj@interchange.ubc.ca and request that your DNA sample be removed from the bank and destroyed.

APPENDIX E: Peer consent

School of Human Kinetics
210, War Memorial Gym
6081 University Boulevard
Vancouver, B.C., Canada V6T 1Z1
Tel: (604) 822-3838 Fax: (604) 822-6842

PEER REVIEW INFORMATION AND CONSENT FORM

Project: The relationship between genetics and skiing/snowboarding behaviours: variants in the Dopamine receptor gene.

Principal investigator: Jim Rupert, **PH.D**.
School of Human Kinetics
Room 346 Wesbrook Building, UBC
Vancouver, B.C., CANADA V6T 1Z2
Phone (604) 822-8462 Fax (604) 822-9451
E-mail: rupertj@interchange.ubc.ca

Other investigators: **Mark Beauchamp, PH.D.;** UBC School of Human Kinetics, Annex
Cynthia Thomson, M.Sc.; UBC School of Human Kinetics

Sponsor: UBC Faculty of Education/School of Human Kinetics

Emergency Telephone Number: Dr Jim Rupert (as above) or cell (778) 386-0908

Introduction: Your friend, from whom you have received this package, has consented to participating in this research project regarding ski/snowboarding behaviours. The goals of the project are to analyze sport behaviours using a ski-specific questionnaire, and a standardized personality questionnaire (ZKPQ); and to analyze whether an association exists between common variants in the dopamine receptor gene (*DRD4*) and sport-specific behaviours and personality types such as sensation seeking/risk taking. The dopamine receptor gene makes the molecule that the neurotransmitter "dopamine" binds to. Neurotransmitters are the chemical signals that the brain uses to connect nerves to cells in the body.

Your participation is voluntary: Your participation is entirely voluntary, so it is up to you to decide whether or not to take part in this portion of the study. Before you decide, it is important for you to understand what the research involves. This information form will tell you about the study, why the research is being done, about your role in the study if you choose to participate.

If you wish to participate, proceed with this information form and follow the instruction for filling out the attached questionnaire regarding your peer from whom you received this package (this should take no longer than 5 minutes). If you do decide to take part in this study it will not affect the status of your friend's participation in the study.

Please take time to read the following information carefully. Feel free to take this form with you to read and contact the researchers at a later date if you wish to participate.

Who is conducting the study: This project is being conducted by researchers in the School of Human Kinetics at UBC. The investigators are not receiving any compensation for doing this research from any commercial organization. You are welcome to request any details concerning the funding arrangements from the Principal Investigator.

Background: A number of studies have implicated the dopamine receptor in the personality/behavioural patterns. Based on twin and family studies, the personality traits are highly hereditary. Common variations in the dopamine receptor gene have been reported over-represented in individuals displaying certain behavioural traits in a number of studies; although the evidence for a functional role for the gene is inconclusive. The genetic contribution to personality has not been studied in a sports context.

What is the purpose of the study?
Objectives: To determine whether there is a genetic association between sport behaviours (in skiing and snowboarding), and genetic variants (alleles) in the gene encoding one of the dopamine receptors (*DRD4*).

The goals of the project in which you are playing a role (and for which your friends has consented):
1) to determine the sport context behavioural patterns for each individual as measured by a ski/snowboarding behaviour questionnaire and to assess personality traits using an established personality questionnaire (ZKPQ);
2) to determine if there is an under/over representation of variants in the *DRD4* among certain groups of skiers/snowboarders.

What does this portion of the study involve?
The questionnaire component involves filling out a brief questionnaire on skiing/snowboarding behaviours of the friend by whom you were recruited.

Please Note: This study will not involve an actual practical ski/snowboard component.

What are the benefits of participating in this study?
There are no benefits for participating as a peer-reviewer in this study.

Will my taking part in this study be kept confidential?
Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. Your answers will be kept confidential from the friend whom you are reviewing. We will not tell the friend who recruited you whether you elected to participate in the study or what your answers to the questions were. No information that discloses your identity will be released or published. Your name will not be kept on the actual questionnaire, so even if there is a security failure, your responses will remain confidential.

If you wish to participate please fill out the following questionnaire, detach it from this consent form (which is for your records) and mail the completed questionnaire to the researchers in the enclosed envelope.

Who do I contact if I want to know more about the study?
You are more than welcome to ask questions about the study at any time. We welcome your comments and suggestions.

Please consider the following points before submitting the completed questionnaire.

I have had sufficient time to consider the information provided and to ask for advice if necessary.
I have had the opportunity to ask questions and have had satisfactory responses to my questions.
I understand that all of the information collected will be kept confidential and that the result will only be used for scientific objectives.
I understand that my participation in this study is voluntary and that I am completely free to refuse to participate, or to withdraw from this study at any time.
I understand that I am not waiving any of my legal rights as a result of signing this consent form.
I have read this form and I freely consent to participate in this study.
I have been given a copy of this for my records.

BY RETURNING A COMPLETED QUESTIONNAIRE, YOU ARE CONSENTING TO BEING A PART OF THE STUDY.

THE QUESTIONNAIRE FOLLOWS ON THE NEXT PAGE.

APPENDIX F: Peer CSSQ-S review form

Genetics of sport-behaviours: Peer Review Form

Name: will peel off this portion **Name of Friend/Participant:** will peel off this portion

(We are only asking you to provide your name to match this questionnaire with an identification code. Once the match has been made, your responses will be made anonymous (coded by ID number) your answers will not be shared with the subject whom you are reviewing.)

PLEASE ANSWER THE FOLLOWING QUESTIONS ABOUT YOUR FRIEND (NOT ABOUT YOURSELF).

Peer's sport of choice: ☐ Skiing ☐ Snowboarding

Peer's level of ability:

☐ Beginner ☐ Novice ☐ Intermediate ☐ Advanced ☐ Expert *
(*any terrain, *any condition)

Average number of runs skied by your friend in a day per difficulty grade:

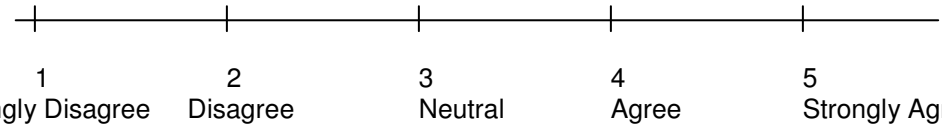
●_____ ■_____ ♦_____ ♦♦_____

How many times have you skied/snowboarded with the subject?
☐ <5 ☐ 5-10 ☐ >10 ☐ Not sure.

Please complete the following questionnaire. It is a sport-specific questionnaire containing 13 specific questions about the skiing/snowboarding behaviours of your friend. Please take your time to read the questions and answer truthfully. There are no right or wrong answers to any of these questions, so please just give your immediate response to the questions. You may feel that in some cases questions are repetitive but please answer every question (unless you feel uncomfortable doing so). Remember that your responses will remain confidential and no one other than the researchers involved in this study will have access to your data.

SECTION I:

Please rate the extent to which you agree or disagree with the following statements. Circle the appropriate answer.



1. He/she likes to ski/ride fast.
   1 2 3 4 5

2. He/she likes to ski/ride down runs that he/she has never been down before.
   1 2 3 4 5

3. He/she likes to start a run even if he/she cannot see what lies ahead (*e.g.* big cornice).
   1 2 3 4 5

4. He/she likes to ski/ride out of bounds.
1 2 3 4 5

5. He/she likes to attempt jumps even if he/she is not sure of the quality of the landing area.
1 2 3 4 5

6. He/she likes to push his/her boundaries when skiing/riding.
1 2 3 4 5

7. If he/she loses control, he/she doesn't seem to immediately slow down, he/she just appears go with it.
1 2 3 4 5

8. If the only way down is a straight line through a narrow pass, he/she would go for it without hesitation even if it means going fast.
1 2 3 4 5

9. He/she is always trying to find new and exciting ways down a run.
1 2 3 4 5

10. A 15-foot high drop off a cliff isn't too high a jump for my friend.
1 2 3 4 5

11. He/she slows down on busy runs.
1 2 3 4 5

12. He/she doesn't slow down on busy runs, instead he/she just appears to dodge people.
1 2 3 4 5
13. If there is a "danger of avalanche" sign, he/she will usually try to find another safer route.
1 2 3 4 5

THANK YOU FOR YOUR TIME. PLEASE PLACE COMPLETED QUESTIONNAIRE (2 pages) IN PRE-STAMPED ENVELOPE.

APPENDIX G: Recipes

Lysis Buffer (DNA Isolation)

100 mM	NaCl
10 mM	TrisCl
25 mM	EDTA
0.5%	SDS

TBE Buffer (5x)

27 g	Tris base (s)
13.75 g	Boric Acid (s)
10 mL	EDTA (0.5 M)
490 mL	Water

TAE buffer (40x), ***Recipe*** *for 4.0 liters, add to 3 litres of water:*

1.6 M Tris	*775.2 grams*
0.52 M acetate	*283.2 gram*
80 mm EDTA	*119.2 grams*

pH to ~8.0 with acetic acid (about 200 mL will be required) and top up with H_2O to a final volume of 4.0 litres

8% PAGE Gel

6.3 mL	Water
2.4 mL	5 x TBE
3.2 mL	Acrylamid/Bis (30%)
80 µL	APS (10%, ammonium persulfate)
12 µL	TEMED

2% Agarose Gel (Volume = 120mL)
120mL 1x TAE Buffer
~2.4 g of Agarose (s)

Microwave approximately 2 minutes until a light boil and solution has turned clear.

***Fsp*I,** New England Biolabs, Beverly, MA, USA

8 µl	PCR product
9.8 µl	Water
2.0 µL	NE Buffer 4
	50mM potassium acetate
	20 mM Tris-acetate
	10 mM magnesium acetate
	1 mM dithiothreitol
0.2 µL	*Fsp*I

Incubate at 37 °C for at least 3 hours.

Standard Polymersase Chain Reaction (1 reaction)

17.2 µL	Water
2.5 µL	10x PCR Buffer
0.9 µL	$MgCl_2$
0.2 µL	dNTP
1.5 µL	F_3 Primer (10 *p*mol/µL)
1.5 µL	R_3 Primer (10 *p*mol/µL)
0.2 U	*Taq* DNA polymerase
1 µL	DNA template

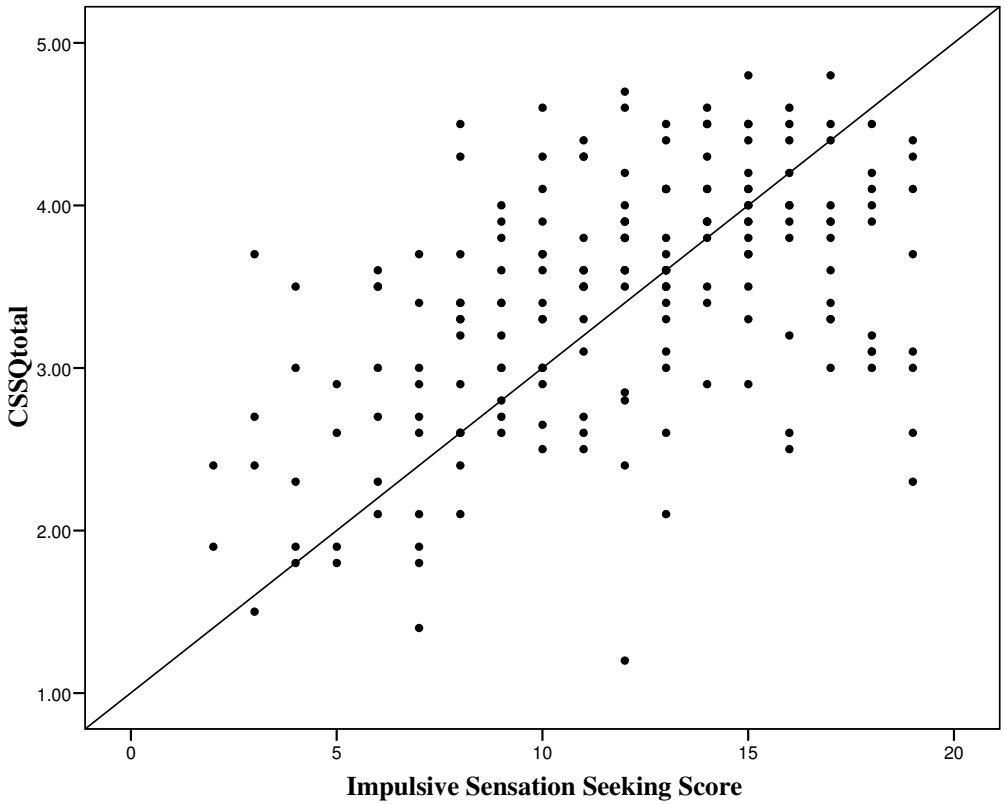
BIORAD (California) **or G-Storm** (Gene Technologies, Essex, UK) **Thermocycler Program**

STEP 1:	98 °C	2 minutes
STEP 2:	98 °C (or 96°C)	45 s
STEP 3:	62 °C	45 s
STEP 4:	72 °C	2 minutes
STEP 5:	Repeat Steps 2 -4, 39 times	
STEP 6:	72 °C	10 minutes
STEP 7:	4 °C	Infinite

APPENDIX H: Optimal PCR conditions for F4/R4 primer pair

The optimal PCR conditions cycled as follows: 95°C for 5 minutes, followed by 39 cycles of 94°C for 45s, 58°C for 45s and 72°C for 2 minutes, finally a chase for 10 minutes at 72°C. The 25 μL reactions contained 20 mM Tris-HCl pH 8.4, 50 mM KCl, 1.8 mM $MgCl_2$, 0.2 μM dNTP, 0.6 μM of each primer, 1 U *Taq*, and 2.0 μL DNA template (approximately 90 ng/μL).

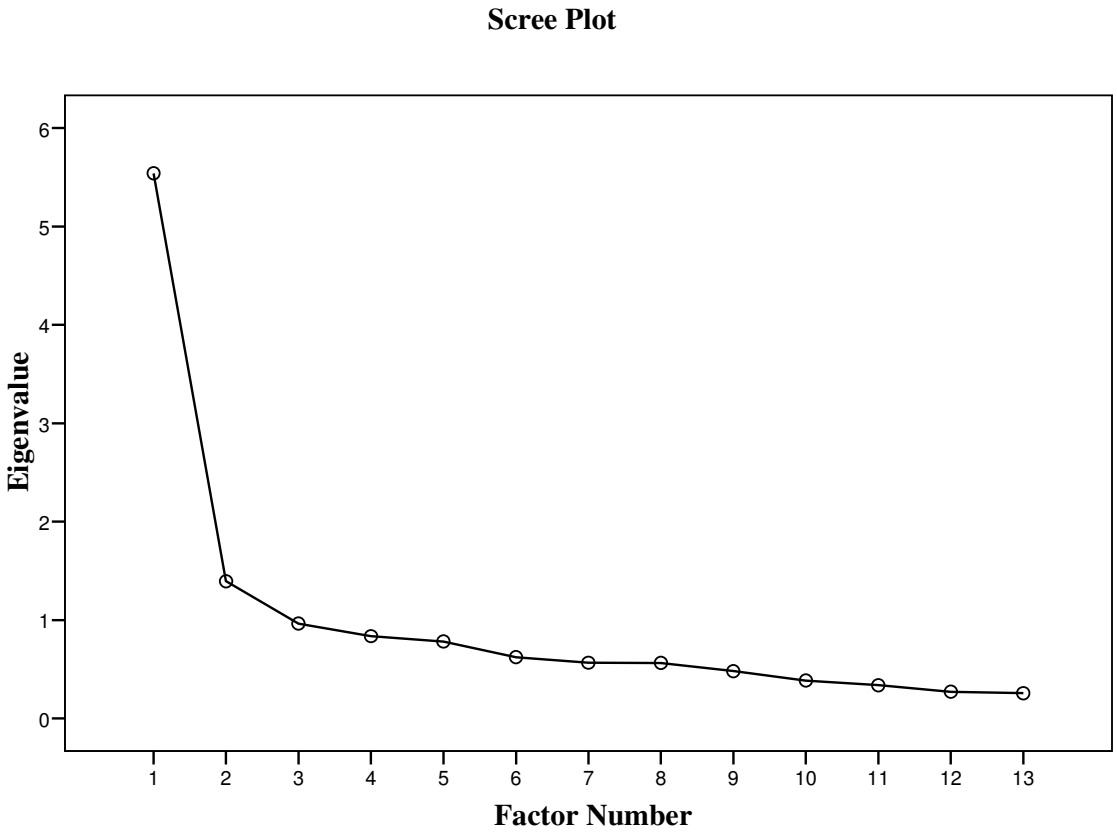
APPENDIX I: A scatter-plot of the total CSSQ scores against the ZKPQ ImpSS scores**.**



APPENDIX J: Normality and descriptive statistics for 13-item CSSQ.

CSSQ Item	Normality		Descriptive Statistics		
	Skewness	Kurtosis	Mean	SD	N
Q1 Speed	-1.26	1.09	4.30	.89	200
Q2 Explore	-1.06	1.69	4.36	.72	200
Q3 Unknown	-.109	-.825	3.18	1.11	200
Q4 Out of Bounds	-.703	-.579	3.65	1.28	200
Q5 Quality of Jump	.484	-.444	2.28	1.03	200
Q6 Push Limits	-.747	.323	3.92	.90	200
Q7 Lost Control	.041	-.843	2.92	1.09	200
Q8 Straight Line	-.172	-.798	3.23	1.15	200
Q9 Novelty	-.734	-.099	3.96	1.00	200
Q10 Cliff Jump	.319	-1.247	2.60	1.38	200
Q11 (Slow Down)	.246	-.799	2.91	1.04	200
Q12 Dodge People	-.054	-.865	3.11	1.12	200
Q13 (Danger Avi)	.412	-.728	2.51	1.16	200

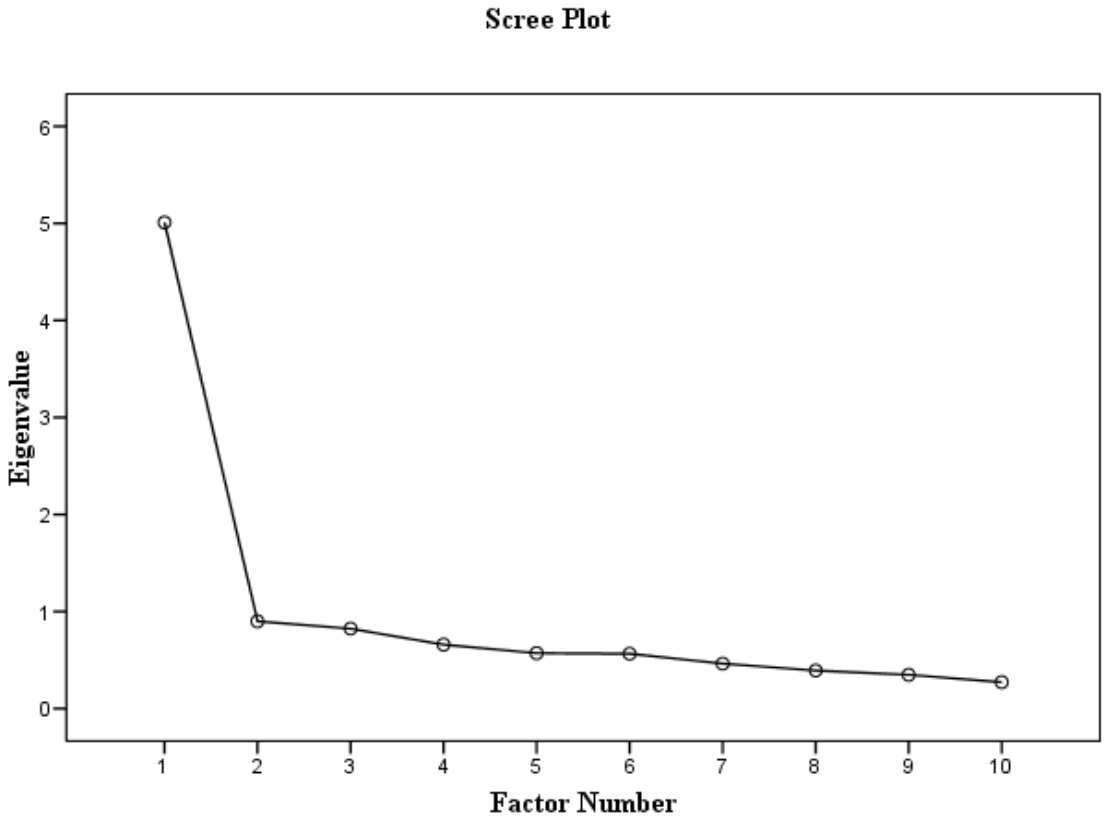
APPENDIX K: Scree plot test representing the percent of variance accounted for by each factor in the FA of the CSSQ**.**



APPENDIX L: Factor analysis of 13-item CSSQ: factor loadings, variances and communalities after ML extraction and varimax rotation.

CSSQ Item	Factor Loadings		
	Factor 1	**Factor 2**	**Communalities**
Q1 Speed	.659		.503
Q2 Explore	.646		.419
Q3 Unknown	.560		.323
Q4 Out of Bounds	.730		.569
Q5 Quality of Jump	.530		.292
Q6 Push Limits	.731		.619
Q7 Lost Control	.540		.33
Q8 Straight Line	.640		.428
Q9 Novelty	.725		.555
Q10 Cliff Jump	.670		.475
Q11 (Slow Down)		.666	.507
Q12 Dodge People		.984	.999
Q13 (Danger Avi)	* < .4		.167
Eigenvalues	4.38	1.78	
% explained variance	33.68	13.67	

APPENDIX M: Scree plot test representing the percent of variance accounted for the contextual sensation seeking factor in the 10-item CSSQ.



APPENDIX N: ANOVA Peer CSSQ and 10-item CSSQ (between groups, df = 1).

CSSQ Item	Sum of Squares	Mean Square	F	p value
Q1 speed	.65	.653	.792	.375
Q2 explore	.40	.395	.806	.371
Q3 unknown	2.06	2.065	1.562	.214
Q4 out of bounds	.40	.395	.217	.642
Q5 quality of jump	3.23	3.226	2.993	.086
Q6 push limits	.20	.202	.267	.606
Q7 lose control	4.27	4.266	3.317	.071
Q8 straight line	.07	.073	.056	.814
Q9 novelty	1.58	1.581	1.565	.213
Q10 cliff jump	.40	.395	.185	.668

APPENDIX O: Pearson's correlation of CSSQ-S and ZKPQ ImpSS

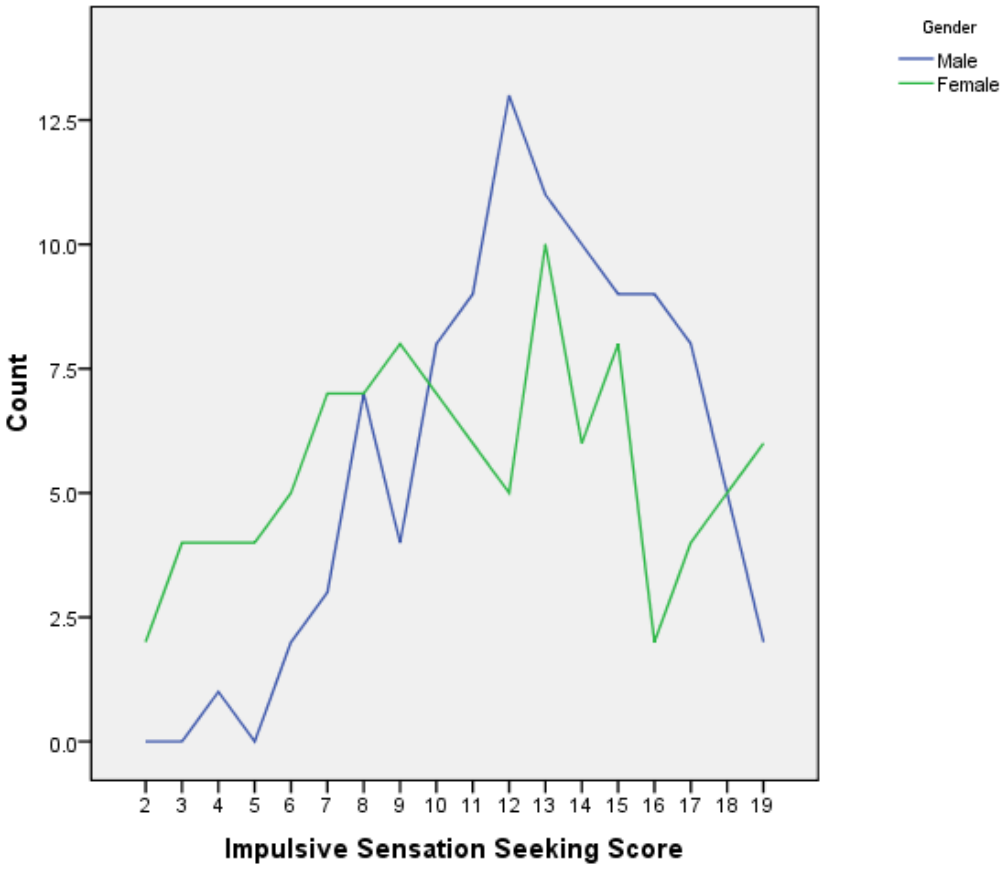
		Total CSSQ-S	**Total ImpSS**
Total CSSQ-S	Pearson Correlation	1	.506**
	Sig. (2-tailed)		.00
	N	194	194
Total ImpSS	Pearson Correlation	.506**	1
	Sig. (2-tailed)	.00	
	N	194	195

** Correlation is significant at the .01 level (2-tailed).

APPENDIX P: Cronbach alphas and tests for normality of ZKPQ subscales using total scores from all subjects (N = 201).

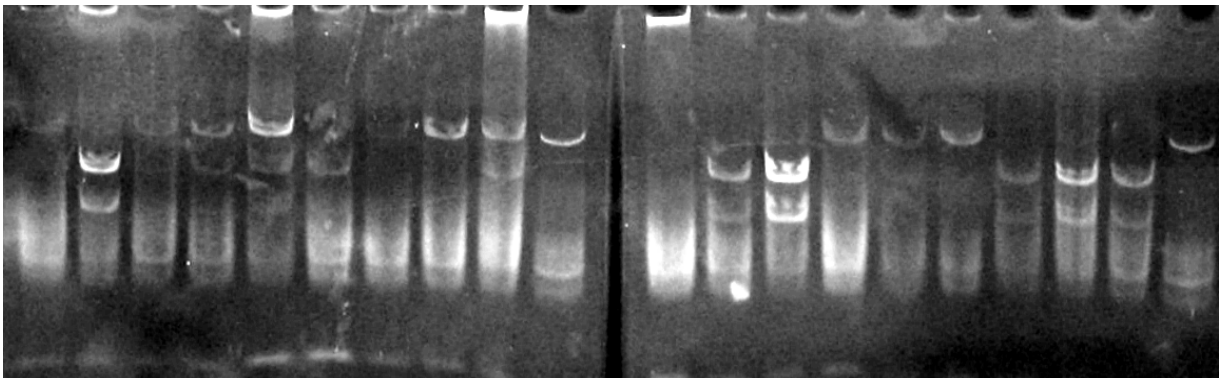
Scale	ImpSS	Agg-Host	Neur-Anx	Soc	Activity
Cronbach Alpha	.834	.708	.828	.757	.994
Mean Inter-item covariance	.040	.025	.039	.033	.024
Mean Inter-item correlation	.207	.120	.203	.157	.924
Skewness	-.270	.089	.813	-.048	-.130
Kurtosis	-.601	-.597	.001	-.578	-.819

APPENDIX Q: Graphed distribution of ImpSS scores for males and females (N = 201).



APPENDIX R: A representative picture depicting digested PCR products and assigned genotypes (16/4/08)

A B



Genotypes from left to right:

A 1-10: C/C▲; T/T; C/T; C/T; C/T; T/T; C/C▲; C/C; C/T; uncut control

B 11-20: n/a; T/T; T/T; C/C; C/C*; C/C; T/T; T/T; T/T; uncut control

Note:

(▲) Lanes 1 and 7 (Photograph A) were unclear and were re-cut and re-photographed to confirm genotypes.

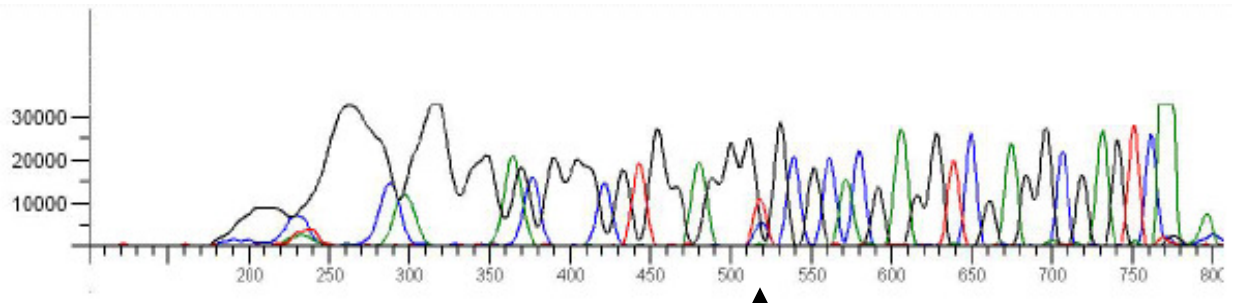
(*) Gel imperfections make lane 15 (Photograph B) difficult to call a C/C or C/T, however re-take photographs do not show evidence of the T allele.

APPENDIX S: Raw data including genotype, ZKPQ ImpSS score, CSSQ score, peer CSSQ (if available).

Subject	Sex	Genotype	ZkPQ ImpSS	CSSQ Total	CSSQ Peer
4	F	C/C	15	4.1	
5	F	T/T	10	3.3	
17	F	C/T	2	1.9	
21	M	C/C	15	4	
27	F	C/T	16	2.5	2.7
28	M	C/T	8	2.9	
31	F	C/C	19	3	
35	M	C/T	18	4	
40	F	C/C	15	3.5	
45	F	C/C	14	3.9	
46	M	C/T	17	3.6	
57	F	C/C	11	3.6	
58	F	C/C	8	3.7	3.6
59	M	C/C	12	3.9	3.7
61	F	C/T	15	3.9	4.2
62	F	C/C	17	3.3	4
63	M	T/T	10	3	3.1
64	F	T/T	11	2.7	2.9
65	F	C/C	9	3.9	4.5
74	M	C/C	15	3.9	
75	F	C/C	17	3	
76	F	T/T	4	3	
81	M	C/C	19	3.7	3.4
88	M	C/T	10	3.9	
89	M	C/C	8	2.1	1.9
101	F	C/T	12	2.9	
109	F	C/T	7	3	
110	M	T/T	12	4	
115	M	C/C	18	4.2	
116	M	C/C	17	4.4	
117	F	C/C	9	4	
120	M	C/T	16	2.6	
122	F	C/T	6	2.1	1.9
123	M	C/C	13	3.8	3.9
126	F	C/T	3	2.4	2.2
128	F	T/T	14	3.9	

Subject	Sex	Genotype	ZkPQ ImpSS	CSSQ Total	CSSQ Peer
129	M	C/T	14	3.8	
132	M	C/T	14	4.3	
133	F	T/T	10	2.5	
138	M	T/T	18	3.1	
143	M	C/C	13	4.4	
146	M	T/T	16	4	
149	M	C/C	15	4.5	4.1
155	F	C/T	13	2.6	
156	M	T/T	12	3.8	
162	M	C/C	16	3.8	
165	M	T/T	12	3.8	
169	M	C/T	4	3.5	
191	M	C/C	17	3.9	
216	M	C/C	12	4.6	
220	M	C/T	12	3.9	
230	M	C/T	13	3.6	
233	F	C/T	5	1.9	2.1
235	M	T/T	13	3.6	
242	F	C/C	10	2.7	
249	M	C/T	12	4.2	4.6
251	F	T/T	12	2.8	
254	F	T/T	11	3.8	5
258	F	C/C	18	3.9	
259	M	T/T	17	3.4	3.7
261	F	C/T	6	2.7	
263	M	C/C	9	3.2	
264	M	T/T	11	4.3	
270	F	C/T	11	2.6	
274	F	C/C	13	3.5	
276	M	C/T	14	4.1	
279	M	C/T	13	4.1	3.4
281	F	C/C	12	3.5	3.3
287	M	C/T	14	4.1	
297	F	C/T	14	3.4	3.4
305	F	C/C	4	1.8	
316	M	T/T	16	4.5	4.5
331	F	C/T	11	3.3	2.2
340	F	C/T	19	2.3	

APPENDIX T: Sequencing data for 'C22' (CMMT/BCRI DNA Sequencing Core Facility, UBC, Vancouver).

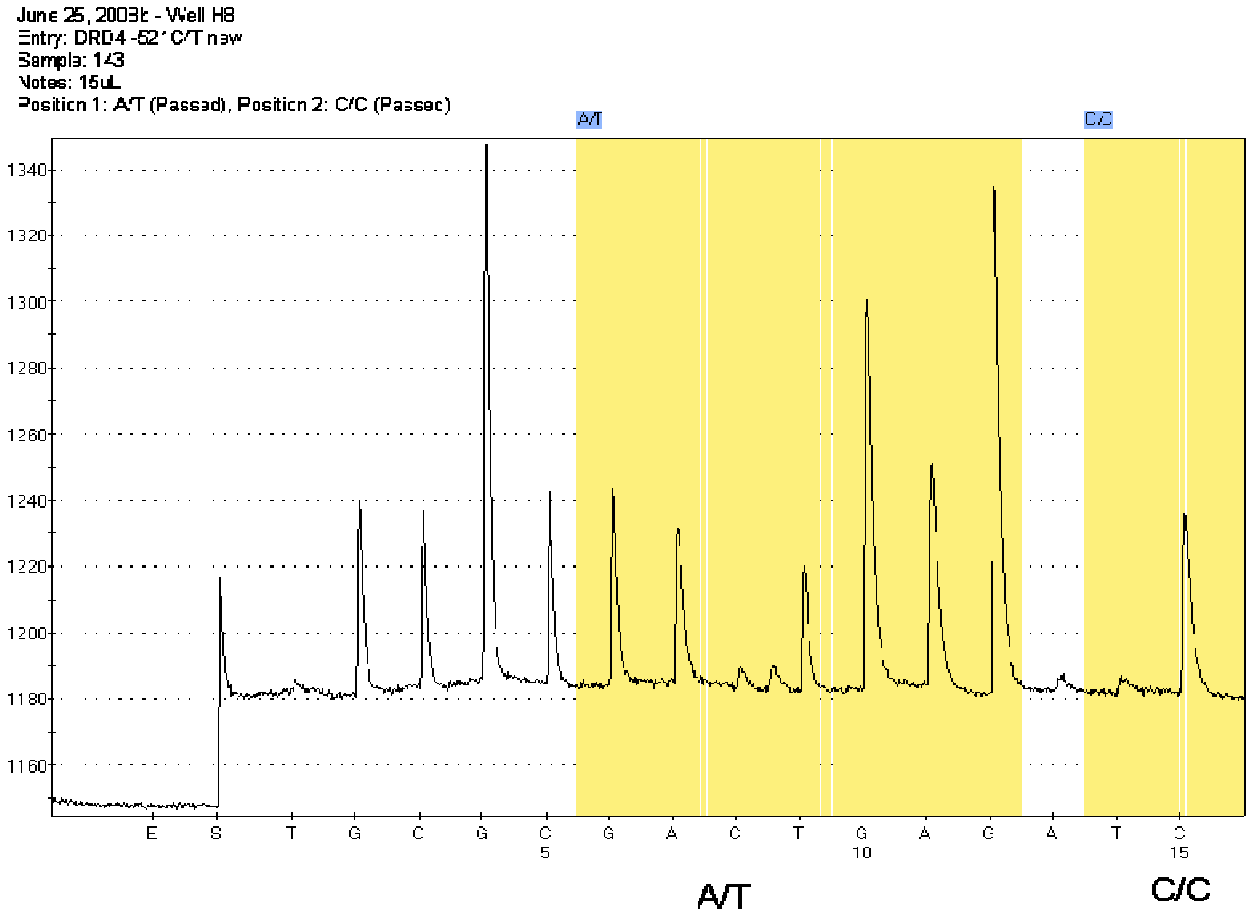


Legend:
Black G
Red T
Green A
Blue C

TGGAGGGTGCGCACGAGGCGAGTCA

-521 C/T

APPENDIX U: Pyrosequencing result. Position 2 shows a C/C genotype, exhibited by the higher peak upon dispensation of the C nucleotide.



APPENDIX V: Expected allele frequencies

Pop 1 = Swedish population (Jonsson et al., 2002): allele C = .42, allele T = .58.
Pop 2 = Caucasian Canadian population (current study): allele C = .58, allele T = .42.

2x2 Contingency Table

Observed Allele Count

	C	T
Pop 1	314	422
Pop 2	86	62

Expected Allele Count

	C	T
Pop 1	333	403
Pop 2	67	81

(Vassar Stats online, http://faculty.vassar.edu/lowry/VassarStats.html)

APPENDIX W: Example of block design for two-way ANOVA using ImpSS scores

	Genotype		
Sex	C/C	C/T or T/T	
Male	15	8	10
	12	18	12
	15	17	18
	19	10	16
	8	16	12
	18	14	12
	17	14	13
	13	4	17
	13	12	11
	15	13	16
	16	12	
	17	14	
	12	13	
	9	14	
	Genotype		
Female	C/C	C/T or T/T	
	15	2	11
	19	16	4
	15	15	14
	14	12	10
	11	7	12
	8	6	11
	17	3	
	9	13	
	17	5	
	9	6	
	10	11	
	18	14	
	13	11	
	12	19	
	4	10	

APPENDIX X: One-way ANOVA blocked by sex.

Sex	Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Observed Power(a)
Male	Genotype	ImpSS	9.70	1	9.70	.92	.345	.02	.15
		CSSQ Total	.15	1	.15	.52	.473	.01	.11
	Error	ImpSS	381.69	36	10.60				
		CSSQ Total	10.50	36	.29				
	Total	ImpSS	7371.00	38					
		CSSQ Total	560.13	38					
Female	Genotype	ImpSS	60.90	1	60.90	3.12	.086	.08	.40
		CSSQ Total	3.08	1	3.08	8.53	.006	.20	.81
	Error	ImpSS	662.74	34	19.49				
		CSSQ Total	12.28	34	.36				
	Total	ImpSS	5235.00	36					
		CSSQ Total	356.99	36					

*significant at alpha = .05, **significant at alpha = .001, (a) computed using alpha = .05
SS – Sum of Squares, η^2 = eta squared measures effect size