# GENETIC COUNSELLING IMPLICATIONS FOR INTERMEDIATE ALLELE PREDICTIVE TEST RESULTS FOR HUNTINGTON DISEASE

by

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## Abstract

Intermediate alleles (IAs) for Huntington disease (HD) have between 27–35 CAG repeats. While they usually do not confer the HD phenotype, they are prone to germline CAG repeat instability. Consequently, offspring are at-risk of inheriting an expanded allele in the HD range (≥36 CAG). Currently there are numerous gaps in our molecular and clinical knowledge on IAs despite their characterization almost 20 years ago. This thesis utilized a unique mixed-method design of molecular and qualitative techniques in order to generate new knowledge on the frequency, haplotype, and CAG repeat instability of IAs and explored current genetic counselling practices and patient understanding and interpretation of an IA predictive test results (PTR).

In the Huntington Disease Biobank at the University of British Columbia, 30% (n=54/181) of IA familial transmissions demonstrated intergenerational CAG repeat instability. Of these unstable transmissions, 14% were repeat expansions into the disease-associated range. In a sample of British Columbia's general population, with no known association to HD, 5.8% (n=92/1594) of individuals were found to have an IA. Of the IAs ascertained in this general population sample, 60% were on haplotypes associated with a high-risk of CAG repeat instability. Paternal CAG-size specific risk estimates for repeat instability, including repeat expansion into the HD range, were established using sperm (n=18763) from 31 males with an IA. Alleles at the upper limits of the intermediate CAG size range (34-35 CAG) had the most significant risk (i.e. 2.5-21.0%) of expanding into the disease range. Interviews with medical genetics service providers and individuals who received an IA-PTR revealed pre-test genetic counselling practices vary based on the individuals' family history and that clients struggled to understand the clinical implications and significance of their IA-PTR.

This thesis substantially contributes to our knowledge of IAs for HD. Collectively the comprehensive findings have important implications for genetic counselling and will help ensure individuals undergoing predictive testing receive appropriate support, education, and counselling on IAs.

## Preface

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I assisted with data collection and performed all the data analysis. I also wrote the manuscript and generated all the tables. Jennifer Collins collected the data and assisted with the analysis.

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I recruited all interview participants with assistance from the respective Medical Genetics Clinics in Canada and Australia. I conducted all interviews, performed all the data analysis, and developed the theoretical model. I also wrote the manuscript and generated all the tables and figures. Lynda Balneaves revised the interview guides and assisted in the data analysis and writing of this manuscript.

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I wrote this manuscript and generated the tables with assistance from Simon Warby and Blair Leavitt.

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*For the advancement of scientific knowledge*
*and clinical care for Huntington disease*

# Chapter 1: Introduction

## 1.1 Huntington Disease

### 1.1.1 Introduction

Huntington disease (HD) is an autosomal dominant, neurodegenerative disorder named after the American physician, Dr. George Huntington, who first characterized the disease in 1872 in a landmark paper entitled *"On Chorea"* [Huntington, 2003]. Huntington wrote this classic paper when he was merely 21 years old, after accompanying his father, who was also a physician, on his medical rounds in East Hampton, New York. During these trips he cared for numerous families suffering from the same debilitating disease and consequently provided a thorough description of its symptoms, hereditary nature, and the fear it instills in families who suffer from it.

> "*The hereditary chorea, as I shall call it, is confined to certain, and fortunately a few families, and has been transmitted to them as an heirloom from generations away back in the dim past. It is spoken of by those in whose veins the seeds of the disease are known to exist, with a kind of horror, and not at all alluded to except through dire necessity, when it is mentioned as "that disorder." It is attended generally by all the symptoms of common chorea, only in an aggravated degree, hardly ever manifesting itself until adult or middle life, and then coming on gradually but surely, increasing by degrees, and often occupying years in its development, until the hapless sufferer is but a quivering wreck of his former self."*

While HD was not described as a separate disease entity until 1872, there is rich historical evidence supporting its presence for many years prior to that time [Bates *et al.*, 2002; Harper, 1991; Hayden, 1981]. In the Middle Ages, a "dancing mania" occurred where an epidemic of people began to suffer uncontrollable dance-like movements. This dancing mania spread to Germany where it was called St. Vitus dance after a young martyr who suffered from this "dancing plague" was put to death in a cauldron of boiling lead and pitch. In Italy, the disease was known as tarantism and was believed to be caused by the bite of a tarantula. Some people also believe that a proportion of the witches killed in the witch trials of Salem, Massachusetts actually had HD and their dance-like movements were mistakenly interpreted as

being possessed by the devil.

### 1.1.2 Epidemiology

HD has been viewed as a relatively uncommon disorder with an overall prevalence of 5-10 per 100,000 [Hayden, 1981]. While it is found world wide, the prevalence of HD varies with ethnicity and geographical location, largely due to founder effects. The most recognized founder effect for HD occurred at Lake Maracaibo in Venezuela, where an immigrant introduced the disease into this small, secluded population over 100 years ago, resulting in an astonishing prevalence of 700 per 100,000 individuals [Hayden, 1981].

Since HD is believed to have major origins in Northern Europe, it is not surprising that populations of Northern European descent are recorded as having the highest prevalence of HD in the world [Hayden, 1981]. In Europe, it was estimated that an average of 4-7 persons per 100,000 are affected with the disease [Harper, 1992; Warby *et al.,* 2011]. Canadian studies suggested 2.4-8.4 persons per 100,000 have HD [Barbeau *et al.,* 1964; Shokeir, 1975]. In the United States, it was estimated that 4.1-5.2 persons per 100,000 are living with the disorder [Folstein *et al.,* 1987; Reed and Chandler, 1958]. The lowest prevalence of HD occurs in Japan and among African and American Blacks, with estimates of 3.8, 1.5, and 0.6 per 1,000,000, respectively [Hayden, 1981; Warby *et al.,* 2011]. Recently, the accuracy of the Northern European prevalence estimates have been called into question given that a study, which examined the number of patients receiving supportive care from HD community organizations in the UK, indicated the disease prevalence is at least 12.4 individuals per 100,000 [Rawlins, 2010; Spinney, 2010]. Consequently, the true prevalence of HD may be underestimated and further epidemiological surveys are needed.

While the disease may be uncommon globally, its significance cannot be overlooked. It is estimated that for every person affected with HD, the disease impacts another 20 individuals, including family members, many of whom are at-risk

for the disorder themselves, friends and caregivers [Huntington Society of Canada, 2011; Aubeeluck, 2008]. Thus, in Canada it is thought that 1 in every 1,000 individuals are touched by HD in some manner.

### 1.1.3 Clinical Features

The clinical features of HD commonly become apparent in mid-adulthood, most often in the third or fourth decade of life but symptoms can onset in juveniles and the elderly [Bates *et al.,* 2002; Harper, 1991; Hayden, 1981]. Juvenile onset occurs prior to age 21 and elderly onset occurs after the sixth decade [McNeil *et al.,* 1997; Nance and Myers, 2001]. Symptoms of the disease gradually progress until death occurs, approximately 15-20 years after the initial diagnosis, most often due to pneumonia, malnutrition, or heart failure. There is no cure for HD and treatment is limited to symptom management.

The symptoms of HD fall into three categories - impaired movement, cognition, and personality. While most patients exhibit deficits in all these areas, the severity and progression of the symptoms can vary, even amongst individuals from the same family. Currently, the presence of specific motor signs is required for a clinical diagnosis of HD, however, cognitive and psychiatric changes often precede the onset of motor dysfunction [Diamond *et al.,* 1992; Duff *et al.,* 2007; Langbehn *et al.,* 2007; Witjes-Ané *et al.,* 2007]. The phenomenon of genetic anticipation also occurs in HD, where the clinical features of the disease have an earlier onset, with increased severity, in successive generations of a family.

The most characteristic symptom of HD is the large, involuntary, jerky movements called chorea [Folstein *et al.,* 1986; Hayden, 1981; Hicks *et al.,* 2008; Kagel and Leopold, 1992; Siemers *et al.,* 1996]. As a consequence of chorea, many patients exhibit an unsteady, erratic gait, which may lead to the perception that the individual is impaired by alcohol. Movement features also include abnormal saccadic eye movements, impaired reflexes, abnormal facial expressions, and difficulties speaking, chewing, and swallowing. Many patients become emaciated due to an

increased caloric requirement generated by the chorea combined with eating difficulties. Symptoms of impaired movement begin gradually and inexorably progress until the individual is completely debilitated.

Individuals affected by HD also experience a progressive decline in their cognitive abilities [Ho *et al.,* 2003; Lawrence *et al.,* 1998; Paulsen and Conybeare, 2005; Podoll *et al.,* 1988; Snowden *et al.,* 2002]. The most significant cognitive impairments involve executive functions, which include one's ability to plan, organize, judge, and think abstractly. As the disease progresses, learning and memory deficits appear and motor speech becomes impaired. The cognitive features of HD often evoke feelings of frustration and sadness, as the patients become aware of their cognitive decline and inability to perform tasks they were previously able to do.

Psychiatric symptoms are present in 30-70% of individuals affected with HD and often occur early in the course of the disease [Berrios *et al.,* 2001; Berrios *et al.,* 2002; Burns *et al.,* 1990; Hahn-Barma *et al.,* 1998; Paulsen *et al.,* 2001]. Psychiatric features primarily include irritability, anxiety, apathy, depression, aggression, and obsessive-compulsive behaviors and thoughts. Psychosis, including delusions, paranoia, and hallucinations, has also been described in a small proportion of patients. The psychiatric features of HD are often described as the most upsetting and challenging aspect of the disease for patients, families, and caregivers [Nordin *et al.,* 1995].

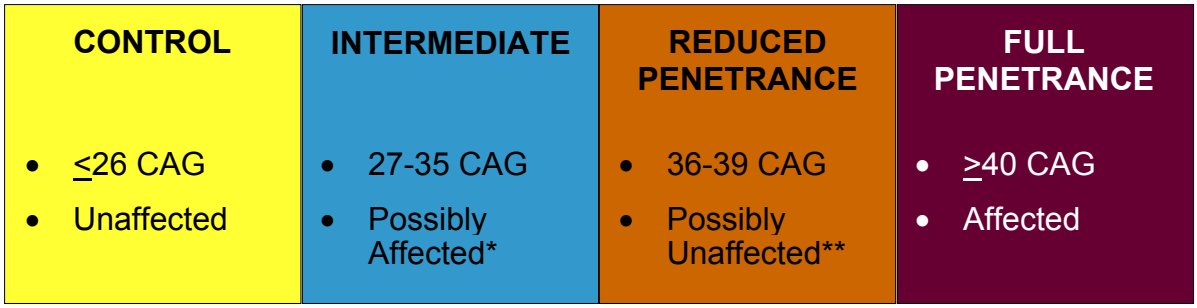
The cardinal neuropathologic feature of HD is atrophy of the caudate nucleus and the putamen, which together constitute the striatum. The atrophy observed is typified by gliosis and neuronal loss of medium spiny neurons [Lange *et al.*, 1976; Vonsattel *et al.*, 1985].

### 1.1.4 Genetics

All humans have two copies of the HD gene (*HTT*), which is found on the short arm of chromosome 4 at 4p16.3 [The Huntington's Disease Collaborative Research Group, 1993]. The *HTT* gene spans 170 kb and contains 67 exons [Ambrose *et al.*, 1994; The Huntington's Disease Collaborative Research Group, 1993]. The *HTT* gene encodes a protein called Huntingtin (HTT), which has 3144 amino acids. While the exact function of the HTT protein is largely unknown, it is essential to early development [Nasir *et al.*, 1995]. HD follows an autosomal dominant inheritance pattern affecting males and female equally. Only one copy of the *HTT* gene with the genetic mutation is required for an individual to develop the disorder. Every child of a person with HD has a 50% risk of also developing the disease, most often when they are adults. In 1993, the genetic mutation responsible for the disease was found to be an expanded cytosine-adenine-guanine (CAG) trinucleotide repeat in exon 1 of the *HTT* gene [The Huntington's Disease Collorative Research Group, 1993]. On average, persons in the general population have 17 CAG repeats in each copy of their *HTT* gene, whereas individuals with HD have 36 or more CAG repeats in one copy of the gene [Kremer *et al.*, 1994].

The number of CAG repeats in the *HTT* gene are divided into four CAG size ranges with different clinical implications for the individual and their offspring. These include control, intermediate, reduced, and full penetrance ranges (Figure 1.1) [ACMG and ASHG, 1998; Potter *et al.*, 2004]. Alleles in the control CAG size range have $\leq$26 CAG repeats and do not impart any clinical consequences for the individual. Intermediate alleles (IAs) have between 27-35 CAG repeats and usually do not confer the HD phenotype for the individual. However, IAs confer a risk for offspring to develop the disease later in life due to germline CAG repeat instability. While HD was once thought to be a classic Mendelian autosomal dominant disorder, where the genotype invariably leads to the phenotype, we now know that the genetic mutation can confer reduced penetrance. HD alleles with 36-39 CAG repeats have incomplete penetrance and are generally associated with a later age of onset, with a proportion of individuals never showing signs of the disorder [McNeil *et al.*, 1997; Rubinsztein

*et al.*, 1996]. Full penetrance HD alleles have ≥40 CAG repeats and impart the characteristic phenotype and age of onset.




* A number of case reports have been published that suggest an intermediate number of CAG repeats caused the HD phenotype

** Reduced penetrance alleles are associated with a late age of onset, with a proportion of individuals never manifesting disease symptoms

**Figure 1.1 CAG Size Ranges in Huntington Disease**

### 1.1.5 CAG Size and Age of Onset

The underlying principle behind the occurrence of elderly and juvenile onset in HD is the strong inverse correlation between age of onset and the number of CAG repeats in the *HTT* gene [Andrew *et al.*, 1993b; Duyao *et al.*, 1993; Nørremølle *et al.*, 1993; Snell *et al.*, 1993]. Generally, a larger CAG repeat size is associated with an earlier age of onset, where individuals with a very large CAG repeat size present with juvenile HD. In contrast, a CAG repeat size at the lower end of the HD range confers reduced penetrance and individuals present with elderly onset, if symptoms occur at all.

The relationship between CAG repeat size and age of onset has been used to make parametric survival models to predict disease onset based on CAG size [Brinkman *et al.*, 1997; Langbehn *et al.*, 2004; Maat-Kievit *et al.*, 2002]. For example, it is predicted that a 40 year-old individual with 42 CAG repeats has an 80% chance of being affected by age 60. At present, these predictions provide limited personalized information about age of onset and severity and progression of symptoms. While the correlation between CAG repeat size and mean age of onset may be discussed in a

clinical setting, the wide confidence intervals of the predictions must be emphasized. Future studies are required to validate these mathematical age of onset models for clinical practice [Langbehn *et al.*, 2010]. The importance of these validation studies is underscored by the fact that the number of CAG repeats in an expanded allele accounts for only 70% of the variation in the age of onset [Brinkman *et al.*, 1997; Langbehn *et al.*, 2004]. Therefore, while CAG repeat size plays a critical role in determining age of symptom onset, it is likely that there are also other *cis or trans* genetic or environmental factors modifying age of onset [Gayán *et al.*, 2008; Li *et al.*, 2007; Nithianantharajah *et al.*, 2008; van Dellen *et al.*, 2005].

## 1.2 Predictive Testing for Huntington Disease

### 1.2.1 Introduction

Prior to the availability of predictive testing for HD, concerns were raised about whether or not it was ethically appropriate to offer such a test when no cure or treatment existed for the disease [Ball and Harper, 1992; Craufurd and Harris, 1986; Terrenoire, 1992; Tyler and Morris, 1990]. These concerns included fear that individuals who received a mutation-positive predictive test result (PTR) would become severely depressed or suicidal; that persons who received a mutation-negative PTR might experience survivor's guilt; and worry that testing could have a negative impact on family relationships [Bates, 1981; Farrer, 1986; European Community Huntington's Disease Collaborative Study Group, 1993; Simpson and Harding, 1993; Wexler *et al.*, 1985]. Conversely, it was also believed that predictive testing could significantly benefit the tested individual by reducing uncertainty, fear, and anxiety, particularly if they receive a mutation-negative result. After careful consideration, HD became the first disease for which predictive testing was offered to at-risk individuals.

In 1986, the first predictive test for HD was performed by linkage analysis using DNA markers mapped to chromosome 4p16.3 [Gusella *et al.*, 1983; Quarrell *et al.*, 1987 Meissen, 1988]. While linkage analysis allowed at-risk individuals to learn with

approximately 95% certainty whether they inherited the HD gene mutation, this method required extensive participation of affected and unaffected family members to establish the segregation of the genetic markers with the disease in the family [Hayden *et al.*, 1988]. Consequently, this requirement excluded some at-risk individuals from testing because family members had died or did not wish to participate [Simpson and Harding, 1993]. Another limitation was the possibility of an incorrect result if a recombination event occurred between the linked markers and the disease gene.

The discovery of the genetic mutation in 1993 replaced linkage analysis with direct-mutation analysis of the number of CAG repeats in the *HTT* gene [The Huntington's Disease Collaborative Research Group, 1993]. Direct-mutation analysis lead to significant improvements to predictive testing and increased the accuracy of the results. It eliminated the possibility of recombination errors and removed the need for extensive family participation. Thus, all at-risk individuals were eligible for testing regardless of their family circumstance [Simpson and Harding, 1993].

In order to be eligible for predictive testing an individual must be the age of majority (i.e. 18 years old), be at either 25% or 50% risk, display no clinical symptoms of the disease, have an established family history of HD, preferably confirmed by genetic testing, be able to provide informed consent and have no major psychiatric disorders or suicidal risks [Benjamin *et al.*, 1994; Fox *et al.*, 1989].

### 1.2.2 Predictive Testing Program

Through consultation with clinicians, scientists, patients and families, and lay support and educational organizations, predictive testing guidelines were established to exemplify best clinical care [Benjamin *et al.*, 1994; Fox *et al.*, 1989; IHA and WFN, 1994]. These guidelines were first developed in British Columbia, Canada and have been subsequently implemented worldwide. While the predictive testing process outlined in the international guidelines signifies best clinical practice, variability in this

process exists amongst different testing centers, most often in regards to the number of genetic counselling sessions offered.

The predictive testing protocol followed at the Centre for Huntington Disease at the University of British Columbia in Vancouver, British Columbia (B.C.) closely adheres to the testing process outlined in the international guidelines. Approximately four genetic counselling sessions are provided in the context of a multidisciplinary health care team including geneticists, genetic counsellors, social workers, and psychologists. The testing process occurs over the course of many weeks to allow the individual sufficient time to assess whether or not they would like to proceed with receiving their PTR. Individuals are encouraged to bring a support person, such as a spouse or a friend, to all genetic counselling sessions. While written informed consent is obtained at the start of the predictive testing process, individuals are frequently reminded that they are free to withdraw from the testing at any time. It is estimated that approximately 25% of individuals who enter the predictive testing process do not receive their PTR [Wiggins *et al.*, 1992].

During the first genetic counselling session, individuals are provided information on the natural history and genetics of HD and the potential benefits and harms of predictive testing [Benjamin *et al.*, 1994; Fox *et al.*, 1989]. The individual's decision making and motives for pursuing predictive testing are explored, as is their available support systems. Individuals are often advised to ensure all life and disability insurance is obtained prior to proceeding with testing. A detailed medical and family history is taken, a psychological assessment is conducted to evaluate the individual's psychological well-being, and a neurological exam is performed to assess whether or not the individual is displaying early signs of HD. Approximately 5-10% of individuals who enter the predictive testing process exhibit symptoms of the disease and thus, genetic testing would be considered diagnostic, not predictive [Hayden and Bombard, 2005]. Individuals are commonly asked if they would like to know the outcome of the neurological exam as it is thought that active involvement

in this decision benefits psychological adjustment to a possible diagnosis [Bloch *et al.*, 1993].

The objective of the second genetic counselling session is to prepare individuals for their PTR [Benjamin *et al.*, 1994; Fox *et al.*, 1989]. During this session, the individual's expectations for their result, the impact and significance a mutation-positive or negative result may have on their life, and strategies for dealing with their result are explored. Individuals are often advised to make a plan for what they will do after receiving their result and are encouraged to think about their plans for disclosing their result to family and friends.

In the third genetic counselling session the individual is provided their PTR in the most clear and direct manner possible [Benjamin *et al.*, 1994; Fox *et al.*, 1989]. Most often, the individual's result is sealed and not known to the medical genetics service providers until moments before the third session begins. Regardless of the result outcome, many individuals experience shock; thus, they are often provided a private moment to digest the information before presented with an opportunity to ask questions.

The fourth genetic counselling session normally occurs two weeks after the individual receives their PTR [Benjamin *et al.*, 1994; Fox *et al.*, 1989]. All individuals receive this follow-up counselling session regardless of their result outcome. The goal of this session is to offer additional counselling and support and provide another opportunity to discuss any questions or concerns. Individuals are reminded that they are welcome to contact their medical genetics providers at any time, for any reason, following the fourth genetic counselling session. In addition to this counselling session, some individuals who receive a mutation-positive PTR may benefit from additional counselling at 6 and 12 months post-result disclosure.

The international predictive testing guidelines recommend that all genetic counselling sessions be conducted in-person, especially disclosure of the PTR

[Benjamin *et al.*, 1994; Fox *et al.*, 1989]. Recently, however, the challenge of providing equitable access to predictive testing for individuals living in rural and remote areas has been highlighted given that testing centers are only located in urban centers [Hawkins and Hayden, 2011; Hawkins *et al.*, 2011]. In British Columbia, a rural predictive testing protocol has been developed for persons who live at a considerable distance from the testing centre in Vancouver. Under this model, individuals are only required to come to Vancouver for their first session, which includes a neurological exam; the remaining sessions are conducted by a physician in the individual's community with the support of the multidisciplinary health care team at the testing centre. While novel mechanisms to improve access to predictive testing have been suggested, including the use of telemedicine and web-based education tools, studies that examine whether accessibility is a barrier to predictive testing and the benefits and drawbacks of novel service delivery methods are needed [Hawkins and Hayden, 2011].

### 1.2.3 Uptake Rates of Predictive Testing

Before predictive testing became available, studies that examined at-risk individuals' intentions regarding the use of predictive testing indicated that a significant proportion, upwards of 80%, would undergo testing [Evers-Kiebooms *et al.*, 1987; Kessler *et al.*, 1987; Mastromauro *et al.*, 1987; Meissen and Berchek, 1987]. However, the actual uptake rate of predictive testing has been considerably lower than expected. Given that predictive testing involves significant psychological and social challenges, only 5-25% of individuals at-risk chose to pursue the test [Creighton *et al.*, 2003; Harper *et al.*, 2000; Laccone *et al.*, 1999; Maat-Kievit *et al.*, 2000]. Currently, Austria and Germany have the lowest rate of predictive testing, <5%, whereas the Netherlands has the highest at 24% [Laccone *et al.*, 1999; Maat-Kievit *et al.*, 2000]. The overall uptake of predictive testing in Canada is 18% but there is variability within the country, with the Maritime provinces having the lowest rate, 12.5%, and British Columbia the highest at 21% [Creighton *et al.*, 2003].

Notably, it has been suggested that the calculation used to determine predictive

testing uptake rates have some inherent errors. More specifically, these calculations often use the cumulative number of persons who have had testing, which is dependent on the number of years testing has been offered at a given testing centre, with a static denominator of individuals at 50% risk, which fails to exclude those who are too young and ineligible for testing [Morrison *et al.*, 2010; Tassicker *et al.*, 2009]. Additional studies that examine the uptake of predictive testing while addressing these calculation errors are needed.

Individuals' motivations for or against predictive testing have been extensively studied [Bloch *et al.*, 1989; Decruyenaere *et al.*, 1995; Evers-Kiebooms *et al.*, 1987; Kessler *et al.*, 1987; Mastromauro *et al.*, 1987; Meissen and Berchek, 1987; Tibben *et al.*, 1993]. Individuals have cited numerous reasons for undergoing predictive testing, including an increased ability to plan for the future and make informed reproductive decisions, the relief of uncertainty and worry, the desire to learn their children's risk status, and simply for the sake of wanting to know. Reasons given for why some individuals prefer not to have the test include concern over the possibility of adverse emotional reactions, fear of receiving a mutation-positive PTR, the preference of living with hope that they will not develop the disease and merely the desire not to know.

The demographic characteristics of individuals who undergo predictive testing have also been extensively documented. Women tend to undergo predictive testing more often than men [Bloch *et al.*, 1989; Creighton *et al.*, 2003; Decruyenaere *et al.*, 1995]. Predictive testing candidates are also older, with a worldwide mean age of approximately 37 years [Almqvist *et al.*, 1999; Creighton *et al.*, 2003]. As a consequence of their older mean age, more individuals receive a mutation-negative rather than positive result because they undergo testing after the average age of symptom onset. Further, a higher proportion of tested individuals have children because they pursue testing after the average childbearing age [Creighton *et al.*, 2003].

### 1.2.4 Psychological Impact of Predictive Testing

The psychological impact of predictive testing has been extensively studied due to fears that testing would have a negative impact on the psychological well-being of the tested individual [Bates, 1981; Farrer, 1986; Wexler *et al.*, 1985]. Initially, it was thought that individuals receiving a mutation-negative PTR would experience the greatest benefit from predictive testing, whereas there was the greatest concern for individuals receiving a mutation-positive PTR [Kessler *et al.*, 1987; Mastromauro *et al.*, 1987; Meissen and Berchek, 1987]. Much to the relief of clinicians, scientists, patients, and families, few of the anticipated negative psychological outcomes have been realized. Irrespective of the result outcome, testing does not appear to negatively impact an individual's long-term psychological well-being [Almqvist *et al.*, 2003; Bloch *et al.*, 1992; Codori and Brandt, 1994; Decruyenaere *et al.*, 2003; Timman *et al.*, 2004; Wiggins *et al.*, 1992]. However, in the short-term, differences in the psychological functioning of individuals who receive either a mutation-positive or negative result have been noted. Individuals who received a mutation-positive result experience the highest level of distress and depression immediately after receiving their result but over time, these levels return to baseline. Several factors were found to influence an individual's response to a mutation-positive result, including a negative coping response to previous life stressors and a history of a psychiatric

disorder [Almqvist *et al.*, 2003; Bloch *et al.*, 1992]. Some individuals found to have a mutation-positive result also became more concerned with their physical well-being, often interpreting normal clumsiness as symptoms of HD [Bloch *et al.*, 1992]. Surprisingly, the frequency of catastrophic events, which include suicide, suicide attempt, or psychiatric hospitalization were rare, being documented in <1% of tested persons [Almqvist *et al.*, 1999]. In fact, predictive testing may decrease suicide rates, which are higher amongst individuals affected by HD compared to the general population [Almqvist *et al.*, 1999; Paulsen *et al.*, 2005].

While the psychological functioning of the majority of individuals who received a mutation-negative PTR improved, over 10% had difficulties coping with their negative result and required additional support [Huggins *et al.*, 1992]. These adverse effects were often encountered when the individual made irreversible decisions, such as having a vasectomy or incurring sizable debt, based on the belief that they would develop HD or had unrealistic expectations about the positive impact a gene-negative result would have on their life. Additionally, it is also thought that some individuals struggled to adjust to their mutation-negative result because it contradicted their conscious or unconscious expectation of the test outcome [Huggins *et al.*, 1992; Kessler and Bloch, 1989]. While survivor guilt was initially thought to be a possible harmful ramification for individuals who will not develop HD, it was not found to be a major cause of impaired psychological functioning [Huggins *et al.*, 1992].

Contrary to previous concerns, the overall impact of predictive testing on individuals' psychological well-being has generally been positive. There are several factors thought to explain this finding. The extensive pre-test counselling process, which explores the motivation for testing, coping strategies, and the availability of support systems, likely excludes individuals more likely to suffer negative consequences [Hayden and Bombard, 2005]. Moreover, it is believed that individuals who pursue predictive testing are a self-selected group that may be more prepared to handle what is perceived to be "bad" news [Bloch *et al.*, 1989; Codori *et al.*, 1994].

However, it is possible that negative coping mechanisms, such as denial, are masking some of the unfavorable psychological effects of predictive testing, particularly for those individuals who have received a mutation-positive PTR [Kessler and Bloch, 1989].

## 1.3 Intermediate Alleles for Huntington Disease

### 1.3.1 Introduction

Since HD was first described over a century ago, it has been portrayed as an inherited illness where only individuals with a family history are at-risk of the disease. On HD's hereditary nature George Huntington wrote:

> *"When either or both of the parents have shown manifestations of the disease… one or more of the offspring almost invariably suffer from the disease if they live to adult age. But if by chance these children go through life without it, the thread is broken and the grandchildren and great-grandchildren of the original shakers may rest assured that they are free from the disease."* [Huntington, 2003]

With our evolving knowledge on the genetics of HD, we know that the genetic thread of HD is not always inherited from generations of past sufferers and it is possible for individuals to develop HD in the absence of a family history [Bateman *et al.*, 1992; Goldberg *et al.*, 1993b]. Shortly after the characterization of the genetic mutation underlying HD, a unique category of HD alleles, termed *intermediate alleles* (IA), were shown to give rise to new genetic mutations for HD. IAs are also referred to as *mutable alleles* [Potter *et al.*, 2004] or *large normal alleles* [Sequeiros *et al.*, 2010].

### 1.3.2 New Mutations

New mutations for HD were initially thought to be exceedingly rare. In fact, the new mutation rate for HD was once predicted to be the lowest of any human genetic disease [Harper, 1991; Hayden, 1981; Vogel and Motulsky, 1979]. Originally, a positive family history of HD was a diagnostic requirement and patients without a family history were explained either by early death of the gene-carrying parent, late onset of HD not recognized by family members, concealment of the disease in the

family, or non-paternity [Bateman *et al.*, 1992]. Today, HD is considered to have one of the highest new mutation rates for any human genetic disease, estimated at ≥10% [Falush *et al.*, 2001]. New mutations are classified using the following criteria: clinical symptoms of HD with molecular confirmation of CAG repeat expansion ≥36 CAG in the *HTT* gene, documentation that the individual's parents were unaffected beyond the characteristic age of onset, and confirmation of paternity [Bateman *et al.*, 1992; Myers *et al.*, 1993].

Since the discovery of the genetic mutation underlying HD, we have learned that the disease can occur in individuals who have unaffected parents. Approximately 8% of patients in Australia, Canada, and Spain had no known family history of the disease and thus, were considered to be new mutations [McCusker, 2000; Almqvist, 2001; Ramos-Arroyo, 2005]. Collectively, these studies support the 10% new mutation rate for HD [Falush *et al.*, 2001]. New mutations are known to arise from IAs and were first identified in unaffected family members, including parents and siblings, of individuals with sporadic HD or *de novo* mutations [Goldberg *et al.*, 1993b]. The discovery of IAs as the cause of new mutations has changed our view of HD such that we can no longer assume the disease is rare outside known pedigrees. Consequently, the possibility of HD now extends to families in the general population who have no history of the disorder.

### 1.3.3 Clinical Implications

IAs for HD have between 27-35 CAG repeats, a range that falls just below the number of repeats required for the disease [ACMG and ASHG, 1998; Potter *et al.*, 2004; Semaka *et al.*, 2006]. Consequently, these individuals will usually not develop HD. However, due to germline CAG repeat instability, the number of CAG repeats may be unstable and increase when the gene is passed to the next generation [Chong *et al.*, 1997; Goldberg *et al.*, 1993b; Goldberg *et al.*, 1995]. This means that offspring are at-risk of inheriting an expanded allele with ≥36 CAG repeats and, thus, may develop HD later in life.

While it is widely believed that IAs do not cause the disease phenotype, there have been some case reports that suggest an intermediate number of CAG repeats caused HD symptoms [Andrich *et al.*, 2008; Groen *et al.*, 2010; Ha and Jankovic, 2011; Herishanu *et al.*, 2009; Kenney *et al.*, 2007]. While some of the genetic, clinical, and neuropathological findings presented in these case reports are suggestive of HD, the symptom presentation varies amongst the cases. Moreover, not all known HD phenocopies or HD-like syndromes were excluded in each case; although it would be challenging to exclude all phenocopies given that the etiology of many phenocopies remains unknown [Wild *et al.*, 2008]. In light of these limitations, these case reports have been critically discussed and the accuracy of the HD diagnosis has been questioned [Reynolds, 2008; Semaka *et al.*, 2008]. Therefore, at present, IAs are not believed to confer the HD phenotype but formal research is needed to explore potential clinical consequences of IAs for the individual in greater detail.

### 1.3.4 Clinical Context

IAs have been identified in two different clinical contexts - in families in which a new mutation for HD has occurred and in families with a long-standing history of HD [Goldberg *et al.*, 1995]. In new mutation families (Figure 1.2.A), IAs are often identified in the parents, most often the father, of a sporadic case of HD. These families have no previous history of the disease and most likely the new mutation was caused by CAG repeat expansion of an IA when passed from parent to child. IAs are also coincidentally discovered in families with a long-standing history of HD in the context of genetic testing (Figure 1.1.B). In this case, the IA is often inherited from the unaffected side of the family, in other words, from an individual who married into the HD family from the general population. In families in which an IA has been identified, HD may appear to have *"skipped"* a generation if an unaffected parent with an IA transmits an expanded allele in the HD range.

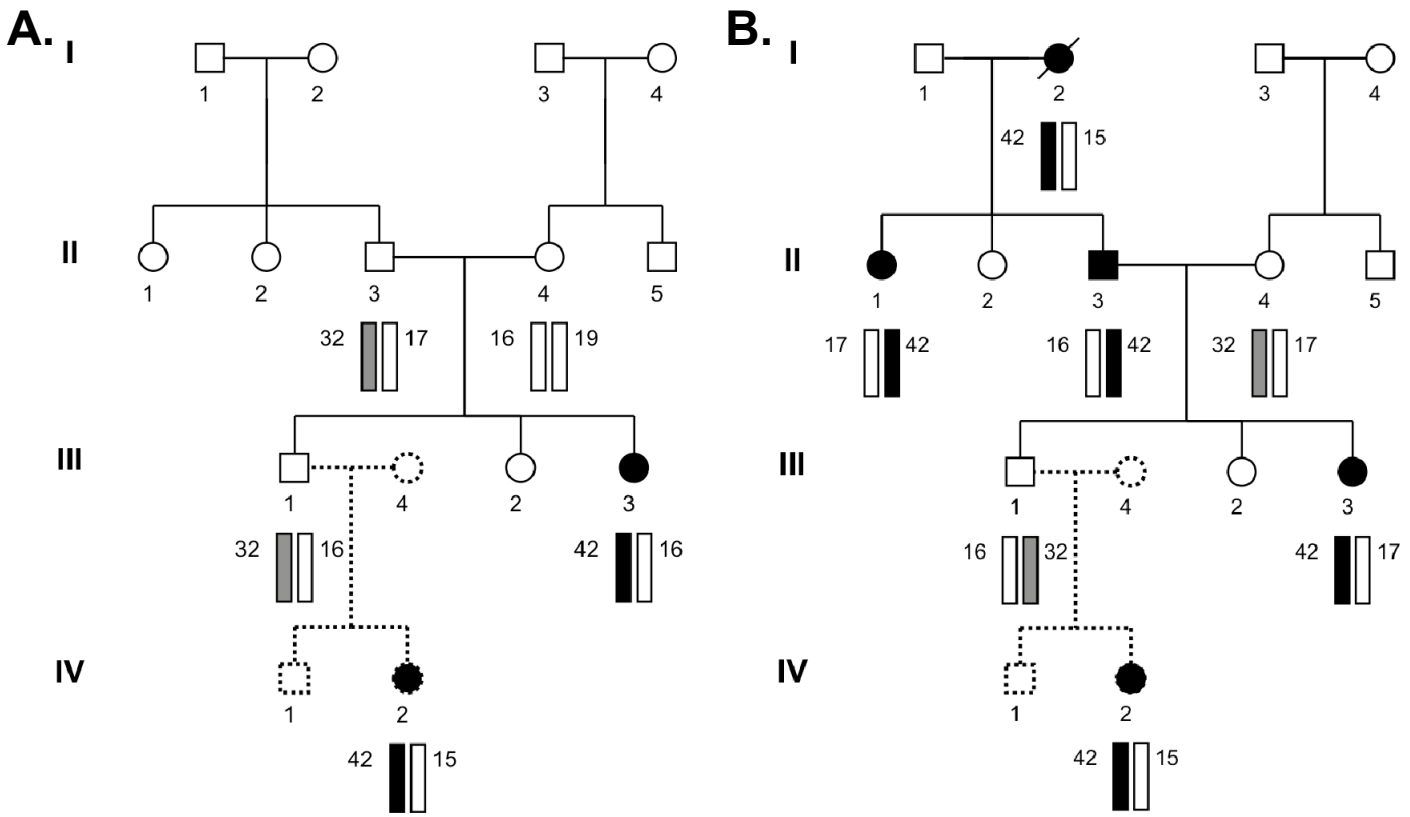



**Figure 1.2 Family Pedigrees Illustrating the Clinical Context in which Intermediate Alleles are Identified**

**A.** New mutation family: Individual III-3 is the first member of the family to be diagnosed with HD. Following the diagnosis, further genetic testing in the family revealed her father (individual II-3) had an intermediate allele (IA). Likely, this IA underwent CAG repeat expansion when passed to individual III-3 causing a new mutation. Individual III-1 was also identified to have received his father's IA but it did not undergo CAG repeat expansion upon transmission. Individual III-1 will likely not develop HD but his children could develop HD later in life if they inherit an expanded IA.

**B.** Family with a long-standing history of HD: Individual III-1 choose to undergo predictive testing because of his long-standing family history – his grandmother (individual I-2), aunt (individual II-1), father (individual II-3), and sister (individual III-3) are affected with the disorder. He did not inherit his father's HD gene but did inherit an IA from his mother (individual II-4). Individual II-4 married into this HD family from the general population but has no history of HD in her biological family. Individual III-1 will likely not develop HD but his children could develop HD later in life if they inherit an expanded IA.

### 1.3.5 Frequency Estimates

There have been a handful of studies examining the frequency of alleles with 27-35 CAG repeats. Published frequency estimates for IAs are variable, ranging from 1.5% to 3.9% [Goldberg *et al.*, 1995; Kremer *et al.*, 1994; Maat-Kievit *et al.*, 2001b; Zühlke *et al.*, 1993]. The variability in the frequency estimates has largely been because the upper and lower limits of the intermediate CAG size range have been redefined over the years as research has shown which CAG sizes confer the disease phenotype and which CAG sizes can expand and produce new mutations [Goldberg *et al.*, 1995; Kelly *et al.*, 1999; Kremer *et al.*, 1994; Maat-Kievit *et al.*, 2001b]. The majority of these frequency estimates were determined by examining the CAG size of control alleles ($\leq$36 CAG) from both affected and unaffected individuals in HD families. Utilizing such clinical samples may result in an ascertainment bias given that IAs in the general population that are not associated with known HD families would not be included. Conversely, these clinical samples may be enriched for IAs from new mutation families and not accurately reflect the frequency of IAs in the general population.

Over the last few years, there have been an increasing number of studies that have examined the occurrence of IAs in samples not associated with HD. Using newborn Guthrie cards, the allelic frequency of IAs was found to be 3.0% in Portugal's general population (n=53/1772 alleles) [Sequeiros *et al.*, 2010]. In a sample of North American ALS patients, the allelic frequency of IAs for HD was found to be 3.2% (n=99/3144 alleles) [Ramos *et al.*, 2012]. These studies indicate a genotypic frequency of approximately 6.0%. In other words, approximately 6.0% of individuals in populations not associated with HD may have an IA. Table 1.1 reports allelic and genotypic IA frequency estimates and the sample populations in which they were determined.

| CAG Size | Country | Sample | Frequency | | Reference |
|---|---|---|---|---|---|
| | | | Allelic | Genotypic | |
| *30-35 CAG | Worldwide | 600 unrelated control chromosomes & 995 lower chromosomes from HD patients | 0.75% (n=15/1595) | -- | (Kremer, 1994) |
| *29-35 CAG | Canada | 746 unrelated control chromosomes & 1727 lower chromosomes from HD patients | 0.93% (n=23/2473) | -- | (Goldberg, 1995) |
| 27-35 CAG | Netherlands | 1101 HD patients and unaffected family members | -- | 3.9% (n=43/1101) | (Maat-Kievit, 2001) |
| 27-35 CAG | Portugal | 886 persons from the general population | 3.0% (n=53/1772) | 6.0% (n=53/886) | (Sequeiros, 2010) |
| | | 146 persons undergoing predictive testing for HD at the genetic counselling clinic | 3.8% (n=11/292) | 6.8% (n=10/146) | |
| | | 1214 samples sent for genetic testing for HD at the diagnostic laboratory | 3.5% (n=85/2428) | 6.8% (n=82/1214) | |
| 27-35 CAG | North America | 1572 ALS patients | 3.2% (n=99/3144) | -- | (Ramos, 2012) |
| | | 4007 lower chromosomes from HD patients | 3.4% (n=138/4007) | -- | |

* Lower frequency estimates likely due to the use of a more narrow intermediate CAG size range

-- Data not reported

**Table 1.1 Intermediate Allele Frequency Estimates**

### 1.3.6 Psychosocial Impact

Numerous studies have examined the psychosocial impact of receiving a mutation-positive or negative PTR. However, there have been no formal studies that examine the predictive testing experience and psychosocial impact of receiving an IA-PTR. Anecdotal evidence suggests that individuals who receive an IA-PTR feel guilt because they will not develop HD yet a risk remains for their children [Maat-Kievit *et al.*, 2001b; van den Boer-van den Berg and Maat-Kievit, 2001]. Feelings of guilt were also evident for individuals who pursued predictive testing in hope of receiving a result that would eliminate their children's risk. Other documented psychological consequences of receiving an IA-PTR include uncertainty about the risk to children and turmoil over informing family members on the non-HD side of the family who are potentially unaware of a risk [Maat-Kievit *et al.*, 2001b; van den Boer-van den Berg and Maat-Kievit, 2001].

## 1.4 CAG Repeat Instability

### 1.4.1 Introduction

The term "dynamic mutation" was coined over 20 years ago to describe the unique category of genetic mutations that are caused by an expanded and unstable repetitive DNA sequence [Richards and Sutherland, 1992]. Dynamic mutations of trinucleotide repeats underlie over 20 devastating neuromuscular or neurodegenerative disorders and microsatellite instability plays an important role in the development of many cancers [Pearson *et al.*, 2005; Vilar and Gruber, 2010; Woerner *et al.*, 2006].

Repeat instability is a characteristic feature of many trinucleotide repeat disorders including fragile X [Hagerman and Hagerman, 2002], myotonic dystrophy [Barceló *et al.*, 1993], Friedreich ataxia [Cossée *et al.*, 1997], dentatorubral-pallidoluysian atrophy [Takano *et al.*, 1998], and spinocerebellar ataxia (SCA) 1 [Chung a*et al.*, 1993], SCA2 [Babovic-Vuksanovic and others 1998], SCA3 [Gu and others 2004], and SCA7 [Gouw *et al.*, 1998]. A characteristic feature of these triplet-repeat

disorders is the phenomenon of genetic anticipation whereby in successive generations of a family, the age of disease onset decreases while the severity of symptoms increases. The molecular basis of anticipation is the inverse correlation between age of onset and disease severity with the number of repeats combined with the propensity for the repeat tract to expand when transmitted from parent to child.

Both germline and somatic CAG repeat instability have been observed in HD [De Rooij *et al.*, 1995; Telenius *et al.*, 1994]. Initially, somatic instability in different tissues, including blood, spleen, liver, kidney, and various regions of the brain, was thought to be rare [MacDonald *et al.*, 1993; Zühlke *et al.*, 1993]. Studies have now revealed that somatic instability occurs in a tissue-specific manner, with the highest levels of repeat instability occurring in the striatum and cerebral cortex, two areas of the brain that undergo neurodegeneration in HD [Kennedy *et al.*, 2003; Telenius *et al.*, 1994]. Based on these findings, it has been suggested that somatic instability itself may be involved in the pathogenesis of the disease [Swami *et al.*, 2009].

Germline CAG repeat instability has been demonstrated in both sperm analyses and familial transmission studies. CAG repeat instability of HD alleles has been shown to be highly biased toward repeat expansions compared to contractions [Duyao *et al.*, 1993; Giovannone *et al.*, 1997; Leeflang *et al.*, 1999; Leeflang *et al.*, 1995; Lucotte *et al.*, 1997; MacDonald *et al.*, 1993; Novelletto *et al.*, 1994b; Ramos *et al.*, 2011; Telenius *et al.*, 1995; Telenius *et al.*, 1994; Trottier *et al.*, 1994; Zühlke *et al.*, 1993]. Germline CAG repeat instability has important clinical implications as it underlies the occurrence of genetic anticipation, which can produce large intra-familial differences in age of onset and symptom severity, and new mutations due to instability of IAs [Kremer *et al.*, 1995; Trottier *et al.*, 1994].

### 1.4.2 Factors Influencing Germline CAG Repeat Instability

Several factors are believed to influence germline CAG repeat instability in HD. These factors include CAG size, the sex and age of the transmitting parent, and the

sequence and haplotype of the allele. The susceptibility of IAs to undergo germline CAG repeat instability is also believed to be influenced by the clinical context in which the allele was identified, specifically whether the IA was ascertained in a new mutation family or was inherited from the general population.

#### 1.4.2.1 CAG Size

CAG size is known to influence the likelihood of germline CAG repeat instability in HD. A strong positive correlation between CAG size and intergenerational repeat instability has been extensively documented [Duyao *et al.*, 1993; Giovannone *et al.*, 1997; Leeflang *et al.*, 1995; Lucotte *et al.*, 1997; MacDonald *et al.*, 1993; Telenius *et al.*, 1995; Telenius *et al.*, 1994; Wheeler *et al.*, 2007; Zühlke *et al.*, 1993]. Studies examining CAG repeat instability have largely focused on control and HD CAG sizes. Control alleles were found to rarely demonstrate intergenerational repeat instability, whereas HD alleles were highly unstable upon transmission from parent to child. Alleles in the intermediate CAG size range were shown to have greater instability than alleles in the control range but do not demonstrate the level of instability observed for HD alleles [Chong *et al.*, 1997; Giovannone *et al.*, 1997; Goldberg *et al.*, 1993b; Goldberg *et al.*, 1995; Leeflang *et al.*, 1995].

#### 1.4.2.2 Sex and Age of Transmitting Parent

Sex of the transmitting parent is another factor found to impact germline CAG repeat instability in HD. IA CAG repeat expansion most often occurs through the male germline [Goldberg *et al.*, 1993b; Kremer *et al.*, 1995; Wheeler *et al.*, 2007]. In fact, all documented cases of new mutations for HD have occurred during paternal transmission of IAs [Goldberg *et al.*, 1993b]. There has only been one documented case of a maternal new mutation - a 33 CAG repeat expanded into an allele with 48 repeats [van Belzen *et al.*, 2009].

The impact of sex of the transmitting parent on CAG repeat instability is also observed when examining large repeat expansions responsible for juvenile HD.

Approximately 80% of these large expansions are paternally inherited [Nance and Myers, 2001; Telenius *et al.*, 1993]. There are only a limited number of juvenile cases due to maternal repeat expansion [Laccone and Christian, 2000; Papapetropoulos *et al.*, 2005].

Studies of HD allele transmissions in families also support a stark difference in the likelihood of CAG repeat instability between paternal and maternal inheritance [Duyao *et al.*, 1993; Kremer *et al.*, 1995; Legius *et al.*, 1994; Nørremølle *et al.*, 1995; Novelletto *et al.*, 1994a; Trottier *et al.*, 1994; Zühlke *et al.*, 1993]. On average, approximately 75% of paternal transmissions demonstrate CAG repeat instability, whereas 60% of maternal transmissions were unstable. Sex also impacts the direction and magnitude of repeat instability with paternal transmissions more likely to undergo large-scale CAG repeat expansions and maternal inheritance more likely to result in smaller repeat contractions. Table 1.2 reports familial transmission studies that have the frequency and magnitude of CAG repeat instability based on parental sex. Notably, the impact of CAG size was not specifically accounted for in these studies. There is also limited data that suggests advanced paternal age (mean 36.7 years) confers a higher risk of CAG repeat instability [Goldberg *et al.*, 1993b], although other studies have not found a significant correlation between paternal age and instability [Giovannone *et al.*, 1997 Wheeler *et al.*, 2007] and additional research is required.

The discrepancy between the rate of paternal and maternal CAG repeat instability in HD is thought to be due to underlying differences in male and female gametogenesis but this remains an understudied area.

| Sex of Transmitting Parent | Number of Transmissions | CAG Repeat Instability of HD Alleles (≥36 CAG) | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | Frequency (%) | | | | Magnitude (+/- CAG) | | |
| | | Stable | Unstable | Contraction | Expansion | Expansion | Contraction | |
| Paternal | 37 | 6 (16.2%) | 31 (83.8%) | 5 (13.5%) | 26 (70.3%) | +1 to +9 | -1 to -3 | (Duyao, 1993) |
| Maternal | 25 | 5 (20.0%) | 20 (80.0%) | 7 (28.0%) | 13 (52.0%) | +1 to +4 | -1 to -4 | |
| Paternal | 32 | 8 (25.0%) | 24 (75.0%) | 3 (9.4%) | 21 (65.6%) | +1 to +28 | -1 to -3 | (Zühlke, 1993) |
| Maternal | 22 | 7 (31.8%) | 15 (68.2%) | 4 (18.2%) | 11 (50.0%) | +1 to +3 | -1 to -3 | |
| Paternal | 34 | 19 (55.9%) | 15 (44.1%) | -- | -- | -- | -- | (Trottier, 1994) |
| Maternal | 31 | 22 (71.0%) | 9 (29.0%) | -- | -- | -- | -- | |
| Paternal | 14 | 2 (14.3%) | 12 (85.7%) | 0 | 12 (85.7%) | +1 to +10 | 0 | (Legius, 1994) |
| Maternal | 7 | 4 (57.1%) | 3 (42.9%) | 2 (28.6%) | 1 (14.3%) | +1 | -- | |
| Paternal | 24 | 5 (20.8%) | 19 (79.2%) | 1 (4.2%) | 18 (75.0%) | +1 to +26 | -1 | (Novelletto, 1994) |
| Maternal | 26 | 7 (26.9%) | 19 (73.1%) | 10 (38. 5%) | 9 (34.6%) | +1 to +3 | -1 to -3 | |
| Paternal | 39 | 7 (18.0%) | 32 (82.0%) | 5 (12.8%) | 27 (69.2%) | -- | -- | (Nørremølle, 1995) |
| Maternal | 26 | 8 (30.8%) | 18 (69.2%) | 9 (34.6%) | 9 (34.6%) | -- | -- | |
| Paternal | 119 | 26 (21.9%) | 93 (78.1%) | 12 (10.1%) | 81 (68.0%) | -- | -- | (Kremer, 1995) |
| Maternal | 135 | 49 (36.3%) | 86 (63.7%) | 34 (25.2%) | 52 (38.5%) | -- | -- | |

-- Data not provided

**Table 1.2 CAG Repeat Instability of Familial Transmissions of Huntington Disease Alleles Based on the Sex of the Transmitting Parent**

#### 1.4.2.3 Haplotype and Sequence

Since the discovery of the genetic mutation underlying HD, numerous studies have identified specific HD haplotypes, defined by a limited number of polymorphisms in the *HTT* gene, including a CCG repeat tract immediately adjacent to the CAG tract and a deletion of three nucleotides, commonly referred to as the delta 2642 codon deletion, which are associated with a higher mean CAG tract length and increased susceptibility to repeat instability [Almqvist *et al.*, 1995; Andrew *et al.*, 1993a; Andrew and Hayden, 1995; Costa *et al.*, 2006; Maat-Kievit *et al.*, 2001a; Rubinsztein *et al.*, 1993a; Rubinsztein *et al.*, 1993b; Rubinsztein *et al.*, 1995; Squitieri *et al.*, 1994]. The CCG repeat length varies between 7-12 repeats in the general population, however, almost all HD chromosomes (93%) have 7 CCG repeats [Andrew *et al.*, 1994]. Further, the delta 2642 deletion is overrepresented on HD chromosomes (38%) relative to normal chromosomes (7%) [Ambrose *et al.*, 1994; Novelletto *et al.*, 1994b].

The availability of high-throughput genotyping has generated new detailed haplotypes using numerous single nucleotide polymorphisms (SNPs) located throughout the HTT gene and surrounding sequence [Lee *et al.*, 2012b; Warby *et al.*, 2009]. One study constructed detailed haplogroups amongst normal, intermediate, and HD alleles [Warby *et al.*, 2009]. Haplogroup ‘A’ was significantly enriched on both HD (95%) and IAs (83%) compared to control alleles (53%). Alleles with a repeat length greater than 26 CAG were 8.4 fold more likely to be on a haplotype A compared to the two other major haplotypes, ‘B’ and ‘C’. Specific haplotype A variants, ‘A1’ and ‘A2’, were also found to be enriched on HD (55% and 29%) and IAs (53% and 33%) compared to control alleles (21% and 26%). Haplotype A1 and A2 were 6.5 and 1.1 fold more likely to be on an expanded CAG allele, respectively. These findings suggest that CAG repeat instability is modulated by haplotype and occurs primarily on these predisposing A1 and A2 haplotypes. Consequently, IAs found on high-risk haplotypes may be more prone to repeat instability than alleles on

a low-risk haplotypes. The impact of haplotype on CAG repeat instability requires further study.

Another study examined whether common genetic variation near the CAG repeat track in the *HTT* gene is associated with differences in the distribution of expanded alleles or the age of disease onset [Lee *et al.*, 2012b]. Seven HD haplotypes were found to account for 83% of the HD alleles examined, however, the age of motor symptom onset was not found to be associated with any of these haplotypes. Based on this data, the authors suggest that *cis*-elements within the *HTT* gene do not modify age of onset in HD and future studies should focus on identifying *trans* genetic modifiers of the disease.

There have been some reports that the 12 base pair sequence between the CAG tract and the CCG tract in the HD gene may acquire point mutations that increase the likelihood of CAG repeat instability [Chong *et al.*, 1997; Goldberg *et al.*, 1995; Kelly *et al.*, 1999]. Two specific point mutations have been identified that lead to a longer CAG or CCG tract with no repeat interruptions. It is possible that the increased purity of this DNA sequence results in greater germline CAG repeat instability as observed in other trinucleotide disorders, such as spinocerebellar ataxia 1 and fragile X [Chong *et al.*, 1995; Eichler *et al.*, 1994]. While these point mutations have been reported in a small number of HD families [Chong *et al.*, 1997; Goldberg *et al.*, 1995; Kelly *et al.*, 1999], they were not found in any control, intermediate, or HD individuals used to construct detailed SNP haplotypes [Warby *et al.*, 2009; unpublished data, Hayden Lab]. Thus, while the absence of these sequence interruptions likely increase CAG repeat instability, these point mutations are not a common factor influencing instability in HD.

#### 1.4.2.4 Clinical Context of Intermediate Alleles

The clinical context in which an IA is identified is thought to impact the likelihood of germline CAG repeat instability. IAs identified in new mutation families were found to demonstrate a higher frequency and magnitude of CAG repeat expansion compared

to similar sized IAs identified on the non-affected side of a family with a long-standing history of HD [Chong *et al.*, 1997; Giovannone *et al.*, 1997; Goldberg *et al.*, 1995; Kelly *et al.*, 1999]. More specifically, a new mutation IA with 35 CAG repeats was found to have a 10% risk of expansion into the HD range, whereas general population IAs of a similar CAG size had a 6% risk [Chong *et al.*, 1997].

It has been suggested that the difference in repeat instability between new mutation and general population IAs may be due to a bias of ascertainment [Semaka *et al.*, 2006]. More specifically, new mutation IAs would be expected to have more instability than general population IAs given that they were clinical ascertained due to CAG repeat expansion into the HD range producing a new mutation. Future research is needed to examine the impact of the IA's clinical context on repeat instability in more detail. Additionally, the relationship between haplotype and these clinical classifications should be examined, as it is possible that the difference in CAG repeat instability may be due to underlying differences in the haplotype of new mutation and general population IAs [Maat-Kievit *et al.*, 2001a].

### 1.4.3 Mechanism of CAG Repeat Instability

The precise mechanism underlying CAG repeat instability in HD remains elusive. It has been speculated that instability may involve both DNA repair and replication, including deficient mismatch repair, DNA polymerase slippage and mispairing, or defective Okazaki fragment processing [Cleary and Pearson, 2005; Lenzmeier and Freudenreich, 2003; McMurray, 2010; Pearson *et al.*, 2005]. Regardless of the exact mechanism of instability, it is widely accepted that the formation of secondary DNA structures, such as hairpin loops, is a critical element in this process. Hairpin loops created by DNA slippage can produce either expansions or contractions depending on whether the loop forms on the nascent or template DNA strand, respectively.

The most commonly cited mechanism for repeat instability is errors that occur during DNA replication. A simple stepwise mutation model, in which the addition or deletion of a single repeat unit (i.e. +/- 1 CAG) during DNA replication due to DNA slippage

has been suggested to underlie repeat instability in HD. However the germline mutation spectrum of intermediate and HD patients obtained by single sperm analysis was found not to fit this model [Leeflang *et al.*, 1999]. In fact, the simple stepwise mutation model poorly captured the observed mutation spectrum, which included repeat expansions greater than one CAG repeat. A model of deficient Okazaki fragment processing during DNA replication was also been tested against the observed mutation spectrum and was found to have a better fit. In this model, secondary structures that form at the 5'-end of Okazaki fragments are thought to result in CAG repeat expansions of variable sizes when not corrected during replication.

Mutations in DNA mismatch-repair genes are known to produce the somatic instability observed in some cancer syndromes, particularly hereditary nonpolyposis colorectal cancer [Vilar and Gruber, 2010]. While one study, which examined the mutational profile of 10 simple tandem repeats loci in colon cancer and HD patients, found that instability was widespread in cancer patients but specific to the HD locus in HD patients suggesting that mismatch-repair does not contribute to repeat instability [Goellner *et al.*, 1997], there is emerging evidence that supports a role for DNA repair mechanisms in repeat instability in HD. Loss of OGG1 or NEIL1, DNA gylcoslases involved in base excision repair, suppresses age-dependent somatic expansion [Kovtun et al., 2007; Mollersen et al., 2012]. Further, deletion of mismatch repair proteins of MSH2 and MSH6 eliminated striatal CAG repeat expansions [Kovalenko et al., 2012].

The preponderance of juvenile HD and new mutation cases resulting from the paternal germline implies that spermatogenesis may play a key role in the molecular mechanism of germline CAG repeat instability in HD. It is not well known when during the process of spermatogenesis instability occurs (i.e. during the mitotic or meiotic cell divisions). Examination of the CAG repeat size in pre-meiotic, meiotic, and post-meiotic testicular germ cells showed that instability occurred before the end of the first meiotic division and some mutations were present before meiosis even

began, suggesting a mitotic origin of instability [Yoon *et al.*, 2003]. Large repeat expansions were also found post-meiotically indicating that instability may occur during and after the meiotic divisions. Determining when during the process of spermatogenesis repeat instability occurs may help illuminate the underlying molecular mechanisms.

### 1.4.4 Quantified Risk Estimates for Germline CAG Repeat Instability

Evidence of CAG repeat instability in HD has primarily been generated using standard PCR approaches. Using this method, a large amount of input DNA produces a smear of multiple unresolved fragments that are thought to be alleles of differing CAG lengths. Densitometric analysis of these PCR products has provided gross quantification of germline instability but cannot provide precise measures, which are required for clinical practice [Giovannone *et al.*, 1997; MacDonald *et al.*, 1993; Telenius *et al.*, 1995; Telenius *et al.*, 1994]. Densitometric scanning of sperm from males with an IA has generated inconsistent evidence of instability, with instability observed in one study but not the other [Giovannone *et al.*, 1997; Telenius *et al.*, 1995].

Familial transmission studies and single sperm analyses have generated some quantified estimates of CAG repeat instability. In families, control alleles were largely stable, with approximately 0.5% of transmissions demonstrating instability [Kremer *et al.*, 1995; Zühlke *et al.*, 1993]. Conversely, HD alleles were unstable in 60-80% of transmissions, of which 10-30% were contractions and 40-60% were expansions [Duyao *et al.*, 1993; Kremer *et al.*, 1995; Legius *et al.*, 1994; Lucotte *et al.*, 1997; Nørremølle *et al.*, 1995; Novelletto *et al.*, 1994a; Telenius *et al.*, 1995; Trottier *et al.*, 1994; Zühlke *et al.*, 1993]. The quantified data on IA repeat instability generated by these familial transmission and sperm studies is limited and inconsistent. These studies are limited by small sample sizes, both in terms of the number of IAs and transmissions/sperm examined. They have also produced inconsistent data on the frequency of IA repeat instability, particularly the risk of expansion into the HD range.

Table 1.3 summarizes the familial transmission studies that have quantified instability of IAs. Briefly, two studies documented significant IA expansion, roughly 30% of transmissions [Goldberg *et al.*, 1993b; Goldberg *et al.*, 1995], whereas two other studies found IAs to be highly stable in transmission [Brocklebank *et al.*, 2009; Sequeiros *et al.*, 2010]. Similar variability was also observed using single sperm analyses with no new mutations observed in one study [Leeflang *et al.*, 1995] but significant expansion into the disease range was seen in another study, between 7.5-20.0% (Table 1.4) [Chong *et al.*, 1997]. Notably, in the latter study, two of the IAs examined had a point mutation in the 12 base pair sequence between the CAG and CCG tract in the HD gene, resulting in a pure CAG repeat tract. It is possible the lack of sequence interruptions in these IAs may explain the higher rates of CAG repeat instability observed.

Statistical modeling based on the incidence of HD, the paternal birth rate, the frequency of *de novo* HD cases, and the frequency of IAs in the general population has also provided numerical risk estimates of IA repeat instability, estimating the probability of a male with an IA having a child who will develop HD later in life is less than 1/1000 [Hendricks *et al.*, 2009].

| CAG Repeat Range | Number of Transmissions | CAG Repeat Instability | | | | | | Reference |
|---|---|---|---|---|---|---|---|---|
| | | Frequency (%) | | | | Magnitude (+/- CAG) | | |
| | | Stable | Unstable | Contraction | Expansion | Contraction | Expansion | |
| Intermediate (29-35 CAG) | 12 | 6 (50.0%) | 6 (50.0%) | 2 (16.7%) | 4 (33.3%) | -1 | +10 to +13 | (Goldberg, 1993) |
| HD (≥36 CAG) | 11 | 3 (27.3%) | 8 (72.7%) | 1 (9.1%) | 7 (63.6%) | -1 | +1 to +16 | |
| New Mutation Intermediate (29-35 CAG) | 22 | 13 (59.1%) | 9 (40.9%) | 2 (9.1%) | 7 (31.8%) | -1 | +1 to +3 | (Goldberg, 1995) |
| General Population Intermediate (29-35 CAG) | 62 | 57 (91.9%) | 5 (8.1%) | 2 (3.2%) | 3 (4.9%) | -1 to -2 | +1 | |
| Intermediate (27-35 CAG) | 69 | 65 (94.2%) | 4 (5.8%) | 3 (4.4%) | 1 (1.4%) | -2 to -6 | +2 | (Brocklebank, 2009) |
| Reduced Penterance (36-39 CAG) | 44 | 26 (59.1%) | 18 (40.9%) | 1 (2.3%) | 17 (38.6%) | -1 | +1 to +11 | |
| Full Penterance (>40 CAG) | 534 | 122 (22.9%) | 412 (77.1%) | 148 (27.7%) | 264 (49.4%) | - | - | |
| Intermediate (27-35 CAG) | 16 | 16 (100.0%) | 0 | 0 | 0 | 0 | 0 | (Sequeiros, 2010) |
| Reduced Penterance (36-39 CAG) | 12 | 5 (41.7%) | 7 (58.3%) | 2 (16.7%) | 5 (41.6%) | -1 to -2 | +1 to +8 | |

**Table 1.3 Quantified Estimates of CAG Repeat Instability of Intermediate and Huntington Disease Allele Familial Transmissions**

| CAG Repeat Range | Number of Sperm Examined | Frequency of CAG Repeat Instability (%) | | | | | Reference |
|---|---|---|---|---|---|---|---|
| | | Stable | Unstable | Contraction | Expansion | Expansion ≥36 CAG | |
| Control (≤28 CAG) | 475 | 472 (99.4%) | 3 (0.6%) | 3 (0.6%) | 0 | 0 | (Leeflang, 1995) |
| Intermediate (29-35 CAG) | 80 | 71 (88.8%) | 9 (11.2%) | 2 (2.5%) | 7 (8.7%) | 0 | |
| Reduced Penetrance (36-39 CAG) | 179 | 46 (25.7%) | 133 (74.3%) | 13 ( 7.3%) | 120 (67.0%) | 0 | |
| Full Penetrance (>40 CAG) | 189 | 3 (1.6%) | 186 ( 98.4%) | 5 ( 2.7%) | 181 (95.7%) | 0 | |
| Control (≤28 CAG) | 459 | 444 (96.7%) | 15 (3.3%) | 11 (2.4%) | 4 (0.9%) | 0 | (Chong, 1997) |
| New Mutation Intermediate (29-35 CAG) | 356 | 190 (53.4%) | 164 (46.1%) | 65 (18.3%) | 101 (28.4%) | 69 (19.4%) | |
| General Population Intermediate (29-35 CAG) | 346 | 221 (63.9%) | 125 (35.3%) | 47 (13.6%) | 78 (22.5%) | 26 (7.5%) | |

**Table 1.4 Quantified Estimates of CAG Repeat Instability Using Single Sperm Analysis**

### 1.4.5 Intermediate Alleles in Other Trinucleotide Repeat Disorders

IAs have been observed in other trinucleotide repeat disorders, including fragile X [Hagerman and Hagerman, 2002], myotonic dystrophy [Barceló *et al.*, 1993], Friedreich ataxia [Cossée *et al.*, 1997], dentatorubral-pallidoluysian atrophy [Takano *et al.*, 1998], and spinocerebellar ataxia (SCA) 1 [Chung *et al.*, 1993], SCA2 [Babovic-Vuksanovic *et al.*, 1998], SCA3 [Gu *et al.*, 2004], and SCA7 [Gouw *et al.*, 1998]. In these disorders, IAs also demonstrate germline repeat instability resulting in new mutations for the disease. In fact, SCA7, caused by an expanded CAG repeat tract, is regarded as the most unstable CAG repeat disorder [Gouw *et al.*, others 1998; Mittal *et al.*, 2005; Stevanin *et al.*, 1998].

In contrast to HD, some disorders, such as fragile X, show a preponderance of maternal instability, likely reflecting an underlying difference in the molecular mechanism [Chonchaiya *et al.*, 2009; Hagerman and Hagerman, 2002; Willemsen *et al.*, 2011]. While not commonly observed in HD, in some disorders the deletion of sequences that disrupt the purity of the repeat tract result in increased instability. For example, in SCA1, the CAG repeat tract of control alleles is interrupted by a CAT repeat, however, these interruptions are lost in both intermediate and disease alleles [Matilla-Dueñas *et al.*, 2008]. Notably, in myotonic dystrophy, fragile X, and Friedreich ataxia, alleles that are susceptible to germline repeat instability are classified as either *intermediate* or *premutation* alleles, with the latter guaranteed to expand into the disease range in the next generation. Table 1.5 provides further details on IAs in other triplet repeat disorders.

| Disease | Inheritance Pattern | Gene/Protein | Repeat | Repeat Size Range | | | Sex of Transmitting Parent Bias | References |
|---|---|---|---|---|---|---|---|---|
| | | | | Control | Intermediate | Disease | | |
| **Spinocerebellar ataxia 1 (SCA1)** | Autosomal dominant | ATXN1 gene/ Ataxin-1 protein | CAG | 6 - 35 [1] | 36 - 38 [2] | 39 - 81 [2] | Paternal: Expansion Maternal: Contraction | (Chung, 1993; Matilla-Dueñas, 2008) |
| **SCA2** | Autosomal dominant | ATXN2 gene/ Ataxin-2 protein | CAG | 15 - 27 [1] | 27 - 34 [2] | ≥ 35 [2] | Paternal: Expansion | (Babovic-Vuksanovic, 1998; Lastres-Becker, 2008) |
| **SCA3 (Machado-Joseph disease)** | Autosomal Dominant | ATXN3 gene/ Ataxin-3 protein | CAG | 12 - 44 [2] | 45 - 60 [2] | 61 - 87 [2] | Paternal: Expansion | (Gu, 2004; Bettencourt, 2011) |
| **SCA7** | Autosomal Dominant | ATXN7 gene/ Ataxin-7 protein | CAG | 4 - 27 [2] | 28 - 35 [2] | 37 - >200 [2] | Paternal: Expansion | (Gouw, 1998; Stevanin, 1998; Mittal, 2005) |
| **Myotonic dystrophy** | Autosomal Dominant | DMPK gene/ Myotonin protein kinase | CTG | ≤ 34 | Premutation: 35-49 | Reduced Penetrance: 50-79<br>Full Penetrance: ≥ 80 | Maternal: Expansion | (Barceló, 1993; Yamagata, 1994; Martorell, 2001) |
| **Fragile X** | X-linked Recessive | FMR1 gene/ Fragile X mental retardation protein | CGG | 5 - 54 [1] | Intermediate: 45 - 60 [2]<br>Premutation: 55 -200 [2] | > 200 [2] | Maternal: Expansion Paternal: Contraction | (Hagerman, 2002; Chonchaiya, 2009; Willemsen, 2011) |
| **Friedreich ataxia** | Autosomal Recessive | FXN gene/ Frataxin protein | GAA | Small: < 12 [1]<br>Large: 12 - 33 [2] | Premutation: 35 - 65 [2] | 66 - 1700 [2] | Paternal: Contraction | (Cossée, 1997; Montermini, 1997; Sharma, 2002) |
| **Dentatorubral-pallidoluysian atrophy** | Autosomal Dominant | ATN1 gene/ Atrophin-1 protein | CAG | 6 - 35 | 36 - 47 | 48 - 93 | Paternal: Expansion | (Takano, 1998; Lee, 2001} |

The purity of the trinucleotide tract influences the likelihood of repeat instability in some disorders: [1] indicates the repeat tract is interrupted; [2] indicates the repeat tract is pure

**Table 1.5 Summary of Other Trinucleotide Disorders with Intermediate Alleles**

## 1.5 Thesis Objectives

The hereditary nature of HD has been a defining feature of the disease since it was first described in 1872 [Huntington, 2003]. With knowledge of the genetic mutation underlying the disease, the risks faced by families affected with HD were thought to be certain - you either inherited your parent's mutation or not. However, since the discovery of CAG repeat mutation, we have learned that HD can occur in the absence of a family history due to CAG repeat instability of IAs. IAs have challenged long-standing beliefs about HD inheritance and extends the risk of HD to families in the general population who have no history of the disorder. With the identification of IAs and their susceptibility to CAG repeat instability, the genetics of HD has become more complex. Consequently, the process of predictive testing and genetic counselling has also become more complicated. Individuals who receive an IA-PTR are now faced with uncertain risks for future generations of their family, in particular, their children and grandchildren. While our knowledge on the molecular pathogenesis of HD has experienced major advances over the years, there remain numerous gaps in our molecular and clinical knowledge on IAs despite their characterization almost 20 years ago.

This thesis utilized a unique mixed-method design of molecular and qualitative techniques, in order to address these large gaps in knowledge about IAs. The ultimate goal of this multidisciplinary thesis was the establishment of new knowledge on IAs that could inform genetic counselling practices regarding IA-PTRs. The following were specific objectives of this thesis:

**1. Determine the frequency and haplotype of IAs in British Columbia's general population**
**2. Examine the haplotype of new mutation and general population IAs**
**3. Establish the frequency and magnitude of IA CAG repeat instability**
**4. Investigate factors influencing IA CAG repeat instability**
**5. Describe current genetic counselling practices regarding IA-PTRs**
**6. Explore individual's understanding and interpretation their IA-PTRs**

# Chapter 2: Unstable Familial Transmissions of Intermediate Alleles in the Huntington Disease Biobank at the University of British Columbia

## 2.1 Synopsis

Quantified risk estimates for IA CAG repeat instability are urgently needed for clinical practice. Data on the likelihood of repeat instability generated from familial transmission studies is limited and inconsistent. The limitations of these studies are primarily due to the use of exceedingly small samples, both in terms of the number of IAs and transmissions examined, and failure to account for factors known to influence repeat instability, particularly CAG size and sex of the transmitting parent. Stark differences in the instability of IA transmissions were also found in these studies. Goldberg and colleagues found IAs to be highly unstable during familial transmission and documented numerous expansions into the disease-associated range [Goldberg *et al.*, 1993a; Goldberg *et al.*, 1993b; Goldberg *et al.*, 1995]. Conversely, in large Venezuelan kindreds, Brocklebank documented no occurrences of repeat expansion into the HD range, although 5.8% (n=4) of transmissions were unstable, either expanding (n=1) or contracting (n=3) within the IA CAG size range [Brocklebank *et al.*, 2009]. Similarly, no expansions into the HD CAG size range were documented in 16 IA transmissions in Portuguese families [Sequeiros et al., 2010]. As several factors are known to influence the risk of CAG repeat instability, variability in any of these aspects may explain the conflicting data.

The purpose of this study was to examine intergenerational CAG repeat instability of IAs for HD using familial transmissions present in the Huntington Disease Biobank at the University of British Columbia (UBC-HD Biobank). The impact of sex, CAG size, and the IA's clinical context (i.e. new mutation or general population) on the frequency and magnitude of instability were also explored. Determining the magnitude and frequency of IA CAG repeat instability, particularly expansion instability, will inform genetic counselling practices regarding IA-PTRs. Defining risk estimates for IA CAG repeat instability is critical to providing accurate information and care. Further, examining which factors modulate the risk of instability is

important for accurate risk assessment during genetic counselling. Quantified risk estimates will also inform individuals' reproductive decision making and may help minimize feelings of uncertainty about the clinical implications of an IA-PTR.

## 2.2 Materials and Methods

We examined the number of IA familial transmissions present in the UBC-HD Biobank. In addition to tissue and DNA, the biobank also contains pedigree and genotype data from all consenting British Columbian families seen for HD diagnostic or predictive genetic testing and a proportion of families that were collected for research purposes.

We examined the intergenerational instability of 181 IA transmissions from 58 unique IAs ascertained from 51 different families. All families were of Northern European descent. For all transmissions, consistent genotyping and/or haplotype data was available to support the segregation of the IA in the family. The available genotyping data for each transmission was variable but included the CCG repeat tract [Andrew *et al.*, 1994], the CA repeat tract [Weber *et al.*, 1993], D4S95 [Andrew *et al.*, 1992], D4S127 [MacDonald *et al.*, 1991] and GT70 [Rommens *et al.*, 1993], and delta 2642 deletion markers [Almqvist *et al.*, 1995]. Detailed SNP haplotype data was also available for a proportion of the transmissions [Warby *et al.*, 2009].

Intergenerational instability was measured as any change in CAG repeat size when the allele was passed from one generation to the next. Transmission data was obtained from both parent-child trios (n=107) and sibships (n=74). For parent-child trios, intergenerational instability was calculated by subtracting the size of the parent's allele from the size of the offspring's allele. For sibships, parental CAG size was inferred to be the CAG size that resulted in the greatest repeat stability within the sibship. More specifically, the most frequent CAG size observed amongst the sibs and/or the CAG size that would result in the fewest number of CAG size changes was assumed to be the parental CAG size. For example, in a sibship with CAG sizes: 32, 33, 33, and 42 the parental CAG size was estimated to be 33 CAG

repeats; thus, assuming two stable transmissions, one -1 CAG contraction, and one +10 CAG expansion. There was no difference ($p>0.05$) in the instability of IA transmissions determined by parent-child trios (25%, n=27/107) or sibships (36%, n=27/74); nor was there a difference ($p>0.05$) in the mean change in CAG size for transmissions determined by parent-child trios (+/-1.14 CAG repeats) or sibships (+/-1.76 CAG repeats).

The statistical analysis of the IA transmissions was largely descriptive in nature. Differences in mean CAG size were assessed using Student's t-test. Chi-square analysis was used to examine differences in the proportion of stable and unstable or contracted and expanded transmissions. A p-value less than 0.05 was considered statistically significant.

### 2.3 Results

A total of 181 IA familial transmissions were examined, of which 30% (n=54/181) demonstrated intergenerational CAG repeat instability (Table 2.1). The majority of unstable transmissions observed were CAG repeat expansions (69%; n=37/54), in contrast to repeat contractions (31%; n=17/54). Of the transmissions that increased in CAG size, 32% (n=12/37) expanded within the IA CAG size range (27-35 CAG), whereas 68% (n=25/37) expanded into the HD range ($\geq$36 CAG). Thus, in this sample, 14% (n=25/181) of IA transmissions resulted in a new mutation for HD. The majority of transmissions that decreased in CAG size contracted within the IA CAG size range (88%, n=15/17), with only a small proportion contracting into the control range ($\leq$26 CAG, 11%, n=2/17). The mean change in CAG size of expanded transmissions (+/- 6.2 CAG repeats) was larger than contracted transmissions (+/-1.3 CAG repeats; $p<0.001$).

Sex of the transmitting parent impacted the stability of IA transmissions in the UBC-HD Biobank. There were 61 paternal and 86 maternal IA transmissions in this dataset (Table 2.2). Sex of the transmitting parent was not known for 34 sibship transmissions. The frequency of repeat instability was greater for male (39%;

n=24/61) compared to female (20%; n=17/86) transmissions ($p<0.01$). Male instability was biased towards expansions (79%; n=19/24) compared to contractions (21%; n=5/24). Conversely, contractions (59%; n=10/17) were more frequent for female transmissions compared to expansions (41%, n=7/17). The mean change in CAG size of male transmissions (+/- 2.3 CAG repeats) was also larger than female transmissions (+/- 0.3 CAG repeats; $p<0.01$). In fact, all IA transmissions that expanded into the HD CAG size range producing a new mutation were paternally inherited. On average paternal expansions increased by 7.2 CAG repeats, whereas the mean change in CAG size of maternal expansions was 1.1 repeats.

CAG size also influenced the stability of IA when passed to the next generation, with alleles at the higher end of the IA CAG size range demonstrating the greatest instability (Table 2.3). In fact, only a single IA (2%; n=1/57) with a CAG size at the lower end of the IA CAG size range (27-29 CAG) expanded beyond the disease threshold in transmission, compared to 19% (n=24/124) of IAs with a CAG size between 30-35 repeats ($p<0.01$). Importantly, of the IAs that expanded into the HD range, 40% (n=10/25) were from IAs with 35 CAG repeats. The repeat expansions from alleles with 35 CAG ranged from +1 CAG to +23 CAG, with a mean change in CAG size of +5 CAG.

The impact of the sex of the transmitting parent was also apparent when examining the instability of IAs with different CAG sizes (Table 2.3). Male transmissions of larger IAs (30-35 CAG, 50%, n=21/45) had higher instability compared to female transmissions of similar sized alleles (16%, n=8/49, $p<0.01$). However, the number of unstable transmissions of IAs at the lower end of the CAG size range (27-29 CAG) was similar between male (19%, n=3/16) and female transmissions (24%, n=9/37, $p>0.05$). Male transmissions of IAs at the upper end of the IA range expanded (86%, n=18/21) more frequently than female transmissions of large IAs (25%, n=2/8, $p<0.01$). In contrast, the impact of sex on the frequency of repeat expansion was not observed for alleles at the lower end of the IA range with the frequency of expanded paternal transmissions (33%, n=1/3) being comparable to

maternal inheritance (55%, n=5/9).

We compared the stability of new mutation (n=76) and general population (n=105) IA transmissions (Table 2.4). IAs transmissions in new mutation families demonstrated substantially greater instability (42%, n=32/76) than general population IA transmissions (20%, n=22/105, $p<0.01$). A total of 96% (n=25/26) of new mutation IA transmissions that increased in CAG size, expanded into the HD range. Conversely, none of the general population IA transmissions (0%, n=0/105) expanded beyond the threshold of repeats required for the disease. The mean change in CAG size of new mutation IA transmissions (+/- 2.97 CAG repeats) was also larger than general population IAs transmissions (+/- 0.25 CAG repeats, $p<0.001$).

When examining the instability of new mutation and general population IAs, the influence of the sex of the transmitting parent was also evident (Table 2.4). Male transmissions of new mutation IAs exhibited greater instability (56%, n=18/32) than male transmissions of general population IAs (21%, n=6/29, $p<0.01$). The stability of female new mutation and general population IA transmissions were comparable (14%, n=2/14 vs. 21%, n=15/72, respectively, $p>0.05$). The mean change in CAG size of transmissions of male new mutation transmissions (+/- 4.22 CAG) was greater than general population transmissions (+/- 0.21 CAG, $p<0.001$). The mean change in CAG size of female new mutation (+/- 0.29 CAG) and general population (+/- 0.26 CAG) transmissions were similar ($p>0.05$).

| | n (% of total) |
|---|---|
| **Number of Transmissions:** | **181** |
| **Stable** | 127 (70%) |
| **Unstable** | 54 (30%) |
| **Expanded** | 37 (21%) |
| Within 27-35 CAG Range | 12 (7%) |
| Into ≥36 CAG Range | 25 (14%) |
| **Contracted** | 17 (9%) |
| Within 27-35 CAG Range | 15 (8%) |
| Into ≤26 CAG Range | 2 (1%) |
| **Mean CAG Size Change of Transmissions:** | **(+/- CAG)** |
| **Total** | 1.4 |
| Expanded | 6.2 |
| Contracted | 1.3 |

**Table 2.1 Summary of the CAG Repeat Instability of Intermediate Allele Familial Transmissions in the Huntington Disease Biobank at the University of British Columbia**

| | n (% of total) |
|---|---|
| **Sex of Transmitting Parent:** | |
| **Male** | **61** |
| Stable | 37 (61%) |
| Unstable | 24 (39%) |
| Expanded | 19 (31%) |
| Contracted | 5 (8%) |
| **Female** | **86** |
| Stable | 69 (80%) |
| Unstable | 17 (20%) |
| Expanded | 7 (8%) |
| Contracted | 10 (12%) |
| **Mean CAG Size Change of Transmissions:** | **(+/- CAG)** |
| **Male** | 2.3 |
| Expanded | 7.2 |
| Contracted | 1.0 |
| **Female** | 0.3 |
| Expanded | 1.1 |
| Contracted | 1.5 |

**Table 2.2 CAG Repeat Instability of Intermediate Allele Familial Transmissions in the Huntington Disease Biobank at the University of British Columbia Based on Sex of the Transmitting Parent**

| | n (% of total) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | **27 CAG** | **28 CAG** | **29 CAG** | **30 CAG** | **31 CAG** | **32 CAG** | **33 CAG** | **34 CAG** | **35 CAG** |
| **Number of Chromosomes** | **10** | **6** | **8** | **7** | **4** | **4** | **6** | **6** | **7** |
| **Number of Transmissions:** | **25** | **11** | **21** | **13** | **15** | **15** | **20** | **14** | **47** |
| **Stable** | 24 (96%) | 6 (55%) | 14 (67%) | 9 (69%) | 12 (80%) | 9 (60%) | 13 (65%) | 8 (57%) | 32 (68%) |
| **Unstable** | 1 (4%) | 5 (45%) | 7 (33%) | 4 (31%) | 3 (20%) | 6 (40%) | 7 (35%) | 6 (43%) | 15 (32%) |
| **Expanded** | 1 (4%) | 1 (9%) | 5 (23%) | 3 (23%) | 3 (20%) | 3 (20%) | 5 (25%) | 6 (43%) | 10 (21%) |
| Within 27-35 CAG Range | 0 | 1 (9%) | 5 (23%) | 0 | 2 (13%) | 2 (13%) | 0 | 2 (14%) | 0 |
| Into the ≥36 CAG Range | 1 (4%) | 0 | 0 | 3 (23%) | 1 (7%) | 1 (7%) | 5 (25%) | 4 (29%) | 10 (21%) |
| **Contracted** | 0 | 4 (36%) | 2 (10%) | 1 (8%) | 0 | 3 (20%) | 2 (10%) | 0 | 5 (11%) |
| Within 27-35 CAG Range | 0 | 2 (18%) | 2 (10%) | 1 (8%) | 0 | 3 (20%) | 2 (10%) | 0 | 5 (11%) |
| Into ≤26 CAG Range | 0 | 2 (18%) | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **Sex of Transmitting Parent:** | | | | | | | | | |
| **Male** | **11** | **2** | **3** | **1** | **6** | **5** | **10** | **4** | **19** |
| Stable | 10 (91%) | 0 | 3 (100%) | 0 | 3 (50%) | 3 (60%) | 5 (50%) | 3 (75%) | 10 (53%) |
| Unstable | 1 (9%) | 2 (100%) | 0 | 1 (100%) | 3 (50%) | 2 (40%) | 5 (50%) | 1 (25%) | 9 (47%) |
| Expanded | 1 (9%) | 0 | 0 | 1 (100%) | 3 (50%) | 2 (40%) | 4 (40%) | 1 (25%) | 7 (37%) |
| Contracted | 0 | 2 (100%) | 0 | 0 | 0 | 0 | 1 (10%) | 0 | 2 (10%) |
| **Female** | **14** | **9** | **14** | **7** | **9** | **8** | **3** | **4** | **18** |
| Stable | 14 (100%) | 6 (67%) | 8 (57%) | 7 (100%) | 9 (100%) | 5 (62%) | 3 (100%) | 2 (50%) | 15 (83%) |
| Unstable | 0 | 3 (33%) | 6 (35%) | 0 | 0 | 3 (38%) | 0 | 2 (50%) | 3 (17%) |
| Expanded | 0 | 1 (11%) | 4 (57%) | 0 | 0 | 0 | 0 | 2 (50%) | 0 |
| Contracted | 0 | 2 (22%) | 2 (20%) | 0 | 0 | 3 (38%) | 0 | 0 | 3 (17%) |

**Table 2.3 CAG Repeat Instability of Intermediate Allele Familial Transmissions in the Huntington Disease Biobank at the University of British Columbia Based on CAG Size and Sex of the Transmitting Parent**

| | n (% of total) | |
|---|---|---|
| | **New Mutation** | **General Population** |
| **Number of Chromosomes:** | 19 | 39 |
| Mean CAG Size | 33 | 30 |
| **Number of Transmissions:** | **76** | **105** |
| **Stable** | 44 (58%) | 83 (80%) |
| **Unstable** | 32 (42%) | 22 (20%) |
| **Expanded** | 26 (34%) | 11(10%) |
| Within 27-35 CAG Range | 1 (1%) | 11 (10%) |
| Into ≥36 CAG Range | 25 (33%) | 0 |
| **Contracted** | 6 (8%) | 11 (10%) |
| Within 27-35 CAG Range | 6 (8%) | 9 (8%) |
| Into ≤26 CAG Range | 0 | 2 (2%) |
| **Sex of Transmitting Parent:** | | |
| **Male** | **32** | **29** |
| Stable | 14 (44%) | 23 (79%) |
| Unstable | 18 (56%) | 6 (21%) |
| **Female** | **14** | **72** |
| Stable | 12 (86%) | 57 (79%) |
| Unstable | 2 (14%) | 15 (21%) |
| **Mean CAG Size Change of Transmissions:** | **(+/- CAG)** | |
| **Total** | 2.97 | 0.25 |
| Expanded | 8.38 | 1.09 |
| Contracted | 1.27 | 1.33 |
| **Male** | 4.22 | 0.21 |
| **Female** | 0.29 | 0.26 |

**Table 2.4 CAG Repeat Instability of New Mutation and General Population Intermediate Allele Familial Transmissions in the Huntington Disease Biobank at the University of British Columbia**

## 2.4 Discussion

This is the largest familial transmission study on IAs reported to date, examining 181 transmissions present in the UBC-HD Biobank. A total of 30% of familial IA transmissions demonstrated intergenerational CAG repeat instability. Repeat expansions were more frequent than contractions, with 14% of expanded IA transmissions causing a new mutation for the disease. This finding is consistent with the 10% new mutation rate reported in the literature [Falush *et al.*, 2001].

The sex of the transmitting parent has been documented as an important factor influencing the likelihood of CAG repeat instability in HD [Kremer *et al.*, 1995; Lucotte *et al.*, 1997]. Not surprisingly, the IA familial transmissions examined in the UBC-HD Biobank support the significant impact of the sex of the transmitting parent on the likelihood of repeat instability. IA repeat instability was most frequent during paternal transmission, in fact, all transmissions that expanded $\geq$36 CAG were inherited from a male. Thus, males with an IA have a significantly higher risk of CAG repeat expansion into the HD range than females.

Strong positive correlations between CAG size and intergenerational repeat instability have been well recognized in the literature [Leeflang *et al.*, 1995 Giovannone *et al.*, 1997; Wheeler *et al.*, 2007]. The IA transmissions examined in the UBC-HD Biobank support the relationship between increasing CAG size and increasing repeat instability. IAs with larger CAG sizes (30-35 CAG repeats) demonstrated greater intergenerational repeat instability, particularly repeat expansion into the HD range, than smaller sized IAs (27-29 CAG). Thus, while 14% of the IA transmissions examined expanded into the HD range, these expansions were mainly from alleles at the upper limits of the IA CAG size range. While alleles with larger CAG repeats are thought to be inherently more unstable, the observed increase in expansions into the HD range may also reflect the fact that for larger IAs, a fewer number of repeats are required to cross the disease threshold. In fact, the mean CAG size change of IA transmissions did not differ based on the allele's CAG size ($p>0.05$). This suggests that while the frequency of repeat instability is

influenced by the IA's CAG size, the magnitude of instability may not be impacted. This data supports the need for CAG size-specific risk estimates for IA instability, especially the risk of expansion into the disease range, in order to provide accurate risk assessment in clinical practice.

Previous reports have suggested there is a difference in the likelihood of repeat expansion for IAs ascertained from new mutation families and those alleles identified on the unaffected side of a family or in the general population [Chong *et al.*, 1995; Giovannone *et al.*, 1997; Goldberg *et al.*, 1995]. It is thought that IAs that have already led to a new mutation have an increased risk of repeat instability compared to IAs that have not previously been shown to expand into the HD range. While new mutation IA transmissions examined in UBC-HD Biobank displayed greater instability than general population IAs, it is possible that this difference may reflect a bias of ascertainment instead of an underlying difference in repeat instability [Semaka *et al.*, 2006]. More specifically, it is not surprising that new mutation IAs have increased instability compared to general population IAs given that they were classified as such based on their documented repeat expansion into the HD range. In other words, the new mutation classification selects for IAs that have demonstrated instability. In fact, if the new mutation IA transmissions that expanded into the disease-associated range (n=25) were excluded, there was no difference in the number of unstable new mutation IA transmissions (86%, n=7/51) compared to general population IAs (21%, n=22/105, $p>0.05$). This finding suggests that the observed difference in instability between these classifications is due to an ascertainment bias. Indeed, the CAG tract of IAs identified in new mutation families and those found in the general population can be both stable and unstable. A total of 58% (n=44/76) new mutation IAs were stable upon transmission and 42% (n=32/76) demonstrated instability. Conversely 79% (n=83/105) of general population IA transmissions were stable and 21% (n=22/105) exhibited instability, albeit not to the extent of causing a new mutation.

The observed difference in repeat instability between new mutation and general population IAs may also be a consequence of CAG size. In total, 94% (n=18/19) of IAs leading to new mutations had a CAG size greater than 29 CAG repeats, compared to 41% (n=16/39) of IAs ascertained from the general population. The mean CAG size of new mutation IAs (33 CAG) was also higher than general population IAs (30 CAG, $p<0.001$). Thus, the discrepancy in repeat instability between these two categories may also be a reflection of their differing CAG size. Given the relationship between increasing instability and increasing CAG size, new mutation IAs may demonstrate greater instability compared to general population alleles given that they have a larger CAG size.

It has also been speculated that the observed difference in repeat instability between new mutation and general population IAs may be due to an underlying difference in haplotype [Maat-Kievit *et al.*, 2001a]. Haplotype analysis has identified specific HD haplotypes that are associated with a higher mean CAG repeat length and more frequent repeat instability [Almqvist *et al.*, 1995; Squitieri *et al.*, 1994]. The majority of new mutation IAs have been found on these common HD haplotypes compared to haplotypes frequently found on control chromosomes [Chong *et al.*, 1995; Goldberg *et al.*, 1995]. Detailed haplotyping, using 22 tagging SNPs throughout the HD gene, have also identified specific HD haplogroups thought to predispose the allele to repeat instability [Warby *et al.*, 2009]. Studies examining the influence of haplotype on the likelihood of IA instability are required. These studies should also explore the relationship between haplotype and the IA's clinical classification. It is possible that new mutation IAs are found more often on haplotypes that pre-dispose to repeat instability compared to general population alleles and thus haplotype may explain the observed increase in instability.

Previous studies that have examined familial transmissions of IAs have generated inconsistent data on their susceptibility to repeat instability. In contrast to the 14% of IA transmissions examined in the UBC-HD Biobank, none of the parent-to-child IA transmissions examined from a 10-generation Venezuelan kindred displayed CAG

repeat expansion into the HD range (n=0/69) [Brocklebank *et al.*, 2009]. Moreover, while 30% of the IA transmissions in the UBC-HD Biobank displayed instability, only 6% (n=4/69) of the Venezuelan transmissions were unstable. Unlike the present study, the number of contractions (n=3/69) exceeded the number of expansions (n=1/69) in the Venezuelan pedigrees. Possible differences in the factors known to impact repeat instability, including sex of the transmitting parent, CAG size, clinical classification of the IA, and haplotype between these two samples may explain the contradictory findings.

Whether disparities in the sex of the transmitting parent between these two samples could explain the different rates of instability is not entirely clear. The single IA transmission that expanded in the Venezuelan kindred was paternally inherited, while all of the contracted transmissions (n=3) were maternal. This sex bias of instability mirrors what was observed in the UBC-HD data, with unstable paternal transmissions biased towards expansion (79%, n=19/24) and maternal instability predisposed to contraction (59%, n=10/17). The impact of CAG size on the differing rates of IA instability found in these two studies is also not apparent. While the exact CAG sizes of the IA transmissions in the Venezuelan kindreds were not provided, it appears that there was a preponderance of alleles sized 27 and 28 CAG. The higher proportion of smaller IA CAG sizes may contribute to the lower frequency of repeat instability observed. The different rates of instability between these studies may also reflect the inclusion of new mutation IA transmissions in the current study, which are selected based on their documented instability into the HD range. Given that none of the Venezuelan transmissions resulted in a new mutation, these IAs would be classified as general population IAs. In an effort to reconcile the different familial transmission data, we compared the repeat stability of the Venezuelan transmission data with only the general population IAs transmission data in the UBC-HD Biobank. However, the frequency of instability still remained higher in the current study (n=22/105) compared to the Brocklebank study (n=4/69, $p<0.01$).

While differences in CAG size, sex of the transmitting parent, and clinical classification of the IA transmissions between these two studies likely contribute to the discrepant instability rates, underlying differences in the haplotype of the study populations could also explain the variability. Haplotype differences between these populations are possible given that the Brocklebank study used large Venezuelan kindreds, composed of related individuals of a Hispanic background, whereas the current study complied transmission data from 51 different families, largely of Northern European descent. Future research is needed to examine the role of *cis* and *trans* genetic and environmental factors that influence CAG repeat instability and the haplotype of different ethnic populations should also be explored.

The inconsistent Venezuelan and UBC-HD Biobank family data may also be a reflection of the small number of IA transmissions examined in each study. Sample sizes of 69 and 181 transmissions, respectively, likely do not have sufficient power to be generalizable. In order to conclusively determine the frequency and magnitude of IA repeat instability, studies utilizing larger sample sizes are required. Beyond increasing the sample size of IA familial transmission studies through international collaborations, the use of sperm analyses would also offer large-scale sample size. Sperm studies would provide more appropriate number of meioses to assess the frequency and magnitude of CAG repeat changes in IAs. While studies of this nature will not assess the risk of maternal CAG repeat instability, this limitation is offset by the fact that the majority of evidence indicates that the risk of IA expansion into the HD range is highest for paternal transmissions [Goldberg *et al.*, 1993b Telenius *et al.*, 1995; Kremer *et al.*, 1995]. Only a single case of a maternal IA expansion into the disease range has been reported [van Belzen *et al.*, 2009].

This study adds to our knowledge on the frequency and magnitude of IA repeat instability and the factors that influence it. However, larger studies are needed and should not only account for known factors that impact repeat instability but also explore other potential genetic and environmental aspects that may play a role in this process. Gaining a more comprehensive understanding of IA repeat instability

and determining accurate and generalizable risk estimates for repeat expansions, particularly into the HD range, will inform clinical practice and help ensure patients receive appropriate education, support, and counselling.

# Chapter 3: High Frequency of Huntington Disease Intermediate Alleles on Predisposing Haplotypes for Repeat Instability in British Columbia's General Population

## 3.1 Synopsis

Published frequency estimates for IAs range from 1.5-6.0% [Goldberg *et al.*, 1995; Kremer *et al.*, 1994; Maat-Kievit *et al.*, 2001b; Ramos *et al.*, 2012; Sequeiros *et al.*, 2010]. However, many of these studies have utilized clinical samples that may bias the frequency estimates generated. Clinical samples may not accurately reflect the true frequency of IAs in the general population not associated with a known HD family. Conversely, the clinical samples may be enriched for IAs that have lead to new mutations. While a growing number of studies have used general population samples, likely resulting in more accurate estimates [Ramos *et al.*, 2012; Sequeiros *et al.*, 2010], there are no studies that have examined the frequency of IAs in a Canadian general population. The first objective of this study is to determine the frequency of IAs in a sample of British Columbia's (B.C.) general population. By determining the frequency of IAs in the general population, the likelihood of identifying an IA through predictive testing can be estimated. Moreover, accurate prevalence estimates will inform genetic counselling practices and ensure comprehensive clinical care.

A second objective of this study was to examine the haplotype of the IAs, including those identified in the general population and new mutation families. There is limited evidence to suggest the clinical context impacts the IA's susceptibility to undergo CAG repeat instability, with IAs ascertained in new mutation families demonstrating greater instability than alleles identified in the general population [Chong *et al.*, 1997; Giovannone *et al.*, 1997; Goldberg *et al.*, 1995; Kelly *et al.*, 1999]. However, it has been suggested that these clinical classifications may be arbitrary and the variability in the instability may be due to underlying differences in haplotype [Maat-Kievit *et al.*, 2001a]. Thus, it may be more accurate to classify IAs by their haplotype instead of the clinical context in which they were ascertained. Examining the haplotype of IAs is also relevant to clinical practice. Clarifying the haplotype of those IAs found in the

general population may shed light on their propensity to expand in future generations. Moreover, establishing whether there is a haplotype difference between general population and new mutation IAs will confirm whether or not it is appropriate to use this factor in clinical risk assessment for instability. This information will inform genetic counselling and also shed further light on the origins and evolution of HD, including the molecular mechanism underlying new mutations.

## 3.2 Material and Methods

### 3.2.1 Sample Populations

We examined 1600 permanently anonymized DNA samples unrelated to HD from individuals in B.C.'s general population. These samples were randomly selected from a larger archived cohort of approximately 6,000 DNA samples. No related individuals were included in the sample, which was identified as being largely composed of persons of Northern European descent. Ethical approval for the use of these archived anonymous samples was obtained from the applicable research ethics boards.

We also examined the IAs present in The Huntington Disease Biobank at the University of British Columbia (UBC-HD Biobank), which includes DNA samples and clinical data from over 2500 consenting British Columbians who underwent either diagnostic or predictive genetic testing for HD and their affected and unaffected family members. A small proportion of the DNA samples in this database were also obtained through international collaborations with other HD researchers from patients and families of particular research interest (i.e. families with early/late age of onset, new mutation families, etc). Consent for the collection, storage, and use of the DNA samples and clinical data was obtained from all individuals and families in the UBC-HD Biobank and the study received ethical approval from the appropriate ethics review committees.

### 3.2.2 CAG Repeat Sizing

The lengths of the "pure" CAG repeat tract (pCAG), the CCG repeat tract, and the "total" CAG tract (tCAG), which includes both the CAG and CCG repeat sequences, were determined [Andrew *et al.*, 1994; Kremer *et al.*, 1995]. Using previously isolated genomic DNA, each repeat tract was amplified by PCR using fluorescently labeled primers that flanked the pCAG, tCAG, or CCG tract. Each pCAG sizing reaction was performed in a reaction volume containing custom forward (HD344F_HEX, 5'-HEX-CCTTCGAGTCCCTCAAGTCCTTC-3', 0.6uM) [Invitrogen, Carlsbad, CA] and reverse (HD450R, 5'-GGCGGCGGTGGCGGCTGTTG-3', 0.6uM) [Invitrogen] primers, in a custom buffer mix designed to assist amplification of the GC-rich repeat region (1X PCR Buffer (10mM Tris-HCl, 50mM KCl, 1.5mM $MgCl_2$, pH 8.3) [Roche, Mannheim, Germany], 3.5% HiDi Formamide [Applied Biosystems, Foster City, CA], 15% glycerol with 0.2 mM each dNTP [Invitrogen] and 1.25 U Roche GMP Grade *Taq* DNA Polymerase [Roche]. 2uL of genomic DNA template, ranging from 20-100 ng/uL, was added to each reaction mix for a total reaction volume of 25uL. Thermocycle conditions consisted of an initial denaturation step of 94°C for 3 minutes, followed by 35 cycles of 94°C, 61°C, and 72°C for 45 seconds each, and a terminal elongation step of 72°C for 5 minutes. PCR products were run by electrophoresis through a 2.5% agarose gel to confirm amplification and approximate size prior to fragment analysis.

Each tCAG and CCG sizing reaction was performed under identical conditions to pCAG sizing in the same custom master mix, but with different forward and reverse primers. For tCAG sizing, the same dye-labeled forward primer was used (HD344F_HEX, 5'-HEX-CCTTCGAGTCCCTCAAGTCCTTC-3', 0.6uM) with a different reverse primer, located downstream of the CCG repeat tract (HD482R, 5'-GGCTGAGGAAGCTGAGGAG-3', 0.6uM). As before, 2uL of genomic DNA template was added to each reaction for a total reaction volume of 25uL, and tCAG products were confirmed on a 2.5% agarose gel prior to fragment analysis.

For CCG sizing, a forward primer immediately upstream of the CCG repeat tract was

used (HD419F, 5'-AGCAGCAGCAGCAACAGCC-3', 0.6uM) with a dye-labeled version of the reverse primer from tCAG sizing (HD482R_6FAM, 5'-6FAM-GGCTGAGGAAGCTGAGGAG-3', 0.6uM). PCR products from tCAG sizing were diluted 100x in $dH_2O$, and 2uL of this dilution used as template for CCG sizing PCR. CCG sizing products were verified by gel electrophoresis on a 3% agarose gel prior to fragment analysis.

PCR products were analyzed using GeneScan fragment analysis on the ABI 3730xl platform, detected using GeneMapper v.4.0 software with GS 500 LIZ internal size standard, and sized relative to controls of known repeat sizes [Applied Biosystems].

### 3.2.3 Haplotype Analysis

IA samples ascertained from B.C.'s general population and those present in the UBC-HD Biobank underwent haplotype analysis based on the study by Warby *et al.* [2009]. Haplotype data was already available for a proportion of the IA samples in the UBC-HD Biobank.

#### 3.2.3.1 SNP Genotyping

Previously isolated genomic DNA from each IA sample was genotyped on a customized Illumina GoldenGate Assay [Illumina, San Diego, CA] at each of 96 SNPs across the HTT gene region, including 22 tagging SNPs defining previously reported haplogroups [Warby *et al.*, 2009]. Control samples from the Warby study were run alongside experimental samples. Specifically, 250 ng of genomic DNA from each sample were converted to active form per manufacturer's instructions, and hybridized to allele-specific and locus-specific oligonucleotides containing universal PCR primer sites. Following extension and ligation between complementary oligos, allele-specific templates representing each SNP site were subjected to universal PCR with Cy3- and Cy5-labeled primers, binding according to the SNP allele present in each template. Products from this universal PCR were then hybridized to Illumina BeadChips by a unique address sequence embedded in each locus-specific oligo,

allowing for separation of universal PCR products by SNP site. Hybridized BeadChips were then scanned on the Illumina HiScan system, yielding relative fluorescence measurements of Cy3 and Cy5 for each SNP in each sample.

Raw fluorescence data for each SNP was analyzed on Illumina GenomeStudio software (V2011.1, Illumina), and clusters automatically assigned to groups of signal intensities representing homozygous and heterozygous genotypes at each SNP site. Automated clusters were manually adjusted to improve call accuracy. Genotype data was successfully generated across 93 SNPs for all samples and controls. There was no clear clustering for the remaining three SNPs, thus they were considered to have failed the analysis and were excluded from haplotype construction.

#### 3.2.3.2 Haplotype Reconstruction

Haplotypes were inferred by a Bayesian algorithm for estimation of most likely SNP sequences among all chromosomes, using PHASE v2.1 [Stephens and Scheet, 2005; Stephens *et al.*, 2001]. Haplotype generation by this method makes no prior assumptions of SNP sequence and, therefore, is not biased toward previously derived haplotypes. Output from PHASE was formatted to yield two complete haplotypes derived from diploid SNP genotypes of each individual sample.

The 22 tagging SNPs (tSNPs) across the HTT gene were used to define three major haplogroups (A, B and C) and 5 haplogroup A variants (A1, A2, A3, A4 and A5). Haplotype A variants 1 and 2 conferred the highest risk for having a CAG-expanded allele [Warby *et al.*, 2009]. Thus, for the purpose of this study, haplotype A variants 1 and 2 are collectively referred to as *high-risk* haplotypes for repeat instability and all other haplogroup A variants and major haplogroups (i.e. B, C, A3, A4, A5, O), which did not confer a high likelihood of a CAG expansion, were collectively referred to as *low-risk* haplotypes.

#### 3.2.3.3 Phasing

IAs from the UBC-HD Biobank were phased using familial trios (i.e. mother, father, offspring) or sibships [Warby *et al.*, 2009]. No familial DNA samples were available to phase those individuals found to have an IA in B.C.'s general population. Therefore, phase for a proportion of these samples (n=49/93) was inferred by phasing CCG repeat size with pCAG repeat size. This is based on the association of major haplotype C with CCG repeat lengths of 8, 9, or 10 compared to major haplotypes A and B, which are consistently associated with the most common CCG length of 7 [unpublished data, Hayden lab]. After haplotype analysis, those samples that were identified as being heterozygous for haplotype C, underwent pCAG, CCG and tCAG sizing. With these three repeat tract lengths, we were able to deduce which CAG repeat size was on the C haplotype based on CCG repeat length.

### 3.2.4 Statistical Analysis

Statistical analysis was largely descriptive in nature. Fisher's exact test was used to examine differences in the proportion of low- and high-risk haplotypes between the difference sample populations using GraphPad Prism Version 5.0A (GraphPad Software, San Diego California USA). A p-value $<0.05$ was considered statistically significant.

## 3.3 Results

### 3.3.1 Frequency of Intermediate Alleles

Of the 1600 DNA samples from B.C.'s general population, CAG sizes were successfully determined for 1594 individuals. The CAG size of the chromosomes (n=3188) ranged from 9 to 38 repeats, with the most common size being 17 CAG. The CAG size distribution of these general population chromosomes is shown in Figure 3.1.

Among all chromosomes sized, 93 IAs were identified, conferring an allelic frequency of 2.9% (Table 3.1). The mean CAG size of IAs in the general population

was 28.9 CAG, with the most frequent size being 27 CAG. There was a preponderance of alleles at the lower end of the intermediate CAG size range (2.3%, 27-30 CAG) compared to upper limits of the range (0.6%, 31-35 CAG). In fact, no IAs with 35 CAG repeats were identified. CAG-size specific frequency estimates for IAs are reported in Table 3.1. Of the 1594 individuals examined, 5.8% (n=92 individuals) or approximately 1 in 17 persons had an IA genotype (Figure 3.1). Interestingly, one individual was found to be a double IA carrier, having a 27/29 CAG genotype.

There were also 6 reduced penetrance HD alleles (36-39 CAG) identified in the sample of B.C.'s general population; two chromosomes sized 36 CAG, three chromosomes sized 37 CAG, and one chromosome sized 38 CAG (Table 3.1). This conferred an allelic frequency of 0.2% . Thus, 0.4% of individuals or approximately 1 in 250 persons in the general population, with no known association with HD, had a reduced penetrance HD genotype (Figure 3.1).

### 3.3.2 Haplotype of Intermediate Alleles

Of the 92 individuals found to have an IA in the sample of B.C.'s general population, 84 were successfully haplotyped. We were able to phase CAG size to haplotype in 58% (n=49) of these samples - 16 samples were phased by haplotype homozygosity and 33 samples were phased by determining pCAG, tCAG, and CCG sizes (see Materials and Methods). Of these general population IAs, 61% (n=30/49) were on high-risk haplotypes for CAG repeat instability (Figure 3.2). We were unable to phase CAG size and haplotype for any of the 6 reduced penetrance alleles identified in the general population sample.

Haplotype data was available for 135 unrelated IAs in the UBC-HD Biobank, of which 71.1% (n=96) were on a high-risk haplotype for repeat instability (Figure 3.2.A). There was no statistical difference in the proportion of IAs on a high-risk haplotype in the general population compared to the clinical sample (p=0.210). Of the IAs in the UBC-HD Biobank, 116 were identified as coming from the general

population and 19 were ascertained in new mutation families. Of the general population IAs, 72.4% (n=84) were on a high-risk haplotype and 63.2% (n=12) of new mutation IAs were also on a predisposing haplotype for repeat instability (Figure 3.2.B). There was no statistical difference in the proportion of high and low risk haplotypes based on the IA's clinical context (p=0.422). Further, when the haplotype data of IAs identified in the sample of B.C.'s general population was added to those general population IAs in the clinic sample, there was still no difference in the proportion of high and low-risk haplotypes (p=0.608) between new mutations and general population IAs. This suggests that other factors may influence the process of repeat instability in these new mutation cases and supports the notion that the increased instability of new mutation IAs is due to a bias of ascertainment that selects for unstable alleles.

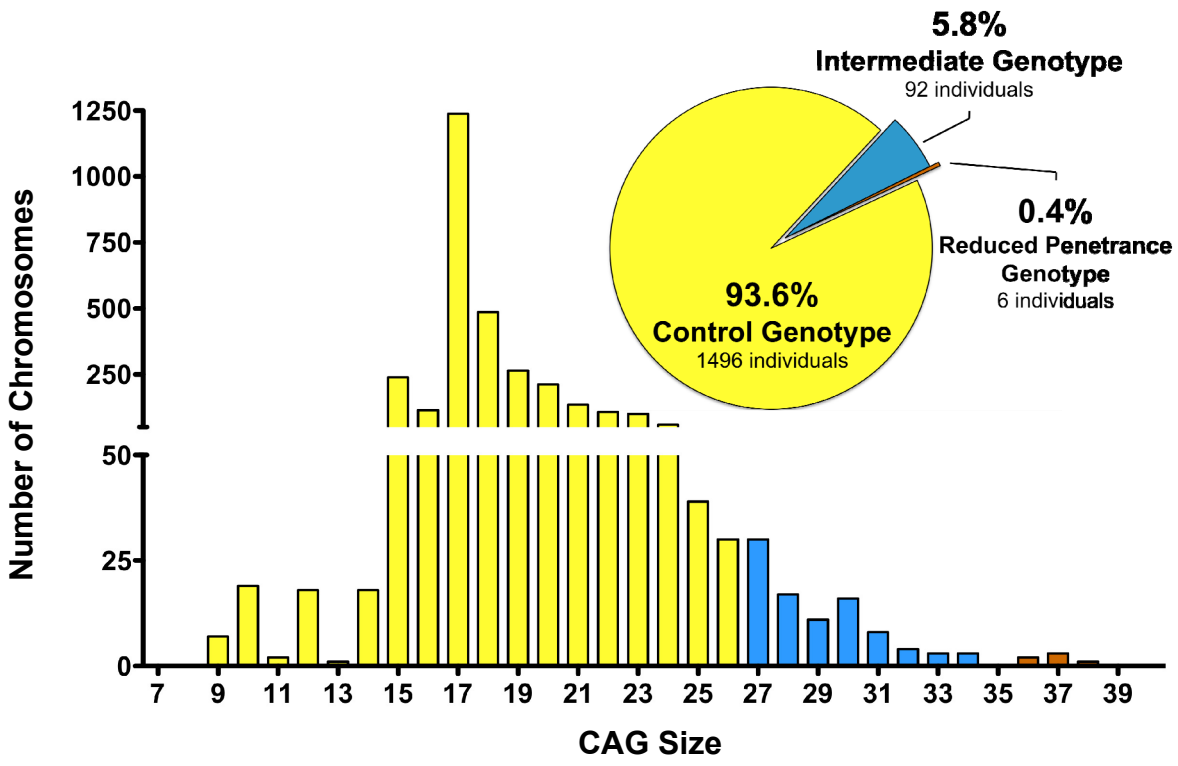



**Figure 3.1 CAG Size Distribution of Chromosomes and Genotypic Frequencies of Individuals in a Sample of British Columbia's General Population**
The CAG size distribution of 3188 chromosomes ascertained from a sample of British Columbia's general population and corresponding genotypic frequencies of 1594 individuals.

| CAG Size | Number of Alleles | Frequency (%) | |
|---|---|---|---|
| | | Allelic | Genotypic |
| **27** | 30 | 0.94 | 1.88 |
| **28** | 17 | 0.54 | 1.08 |
| **29** | 12 | 0.38 | 0.75 |
| **30** | 16 | 0.50 | 1.00 |
| **31** | 8 | 0.25 | 0.50 |
| **32** | 4 | 0.13 | 0.25 |
| **33** | 3 | 0.09 | 0.19 |
| **34** | 3 | 0.09 | 0.19 |
| **35** | 0 | 0.00 | 0.00 |
| **Intermediate Total** | **93** | **2.92** | **5.83** |
| **36** | 2 | 0.07 | 0.14 |
| **37** | 3 | 0.09 | 0.19 |
| **38** | 1 | 0.03 | 0.06 |
| **39** | 0 | 0.00 | 0.00 |
| **Reduced Penetrance Total** | **6** | **0.19** | **0.38** |

**Table 3.1 CAG-Size Specific Frequency Estimates for Intermediate and Reduced Penetrance Alleles in a Sample of British Columbia's General Population**

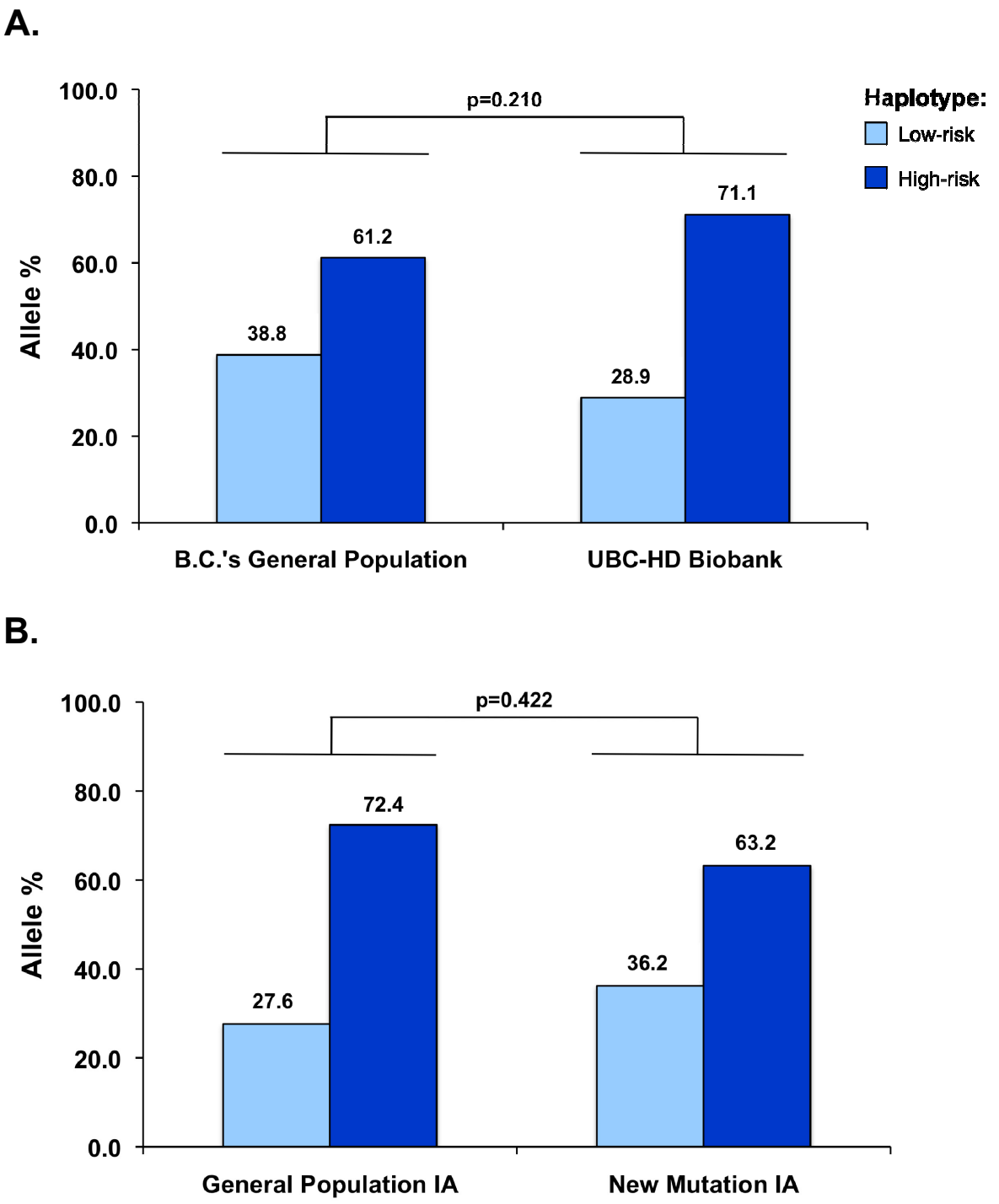



**Figure 3.2 Proportion of Intermediate Alleles on Haplotypes with Low and High Risk for CAG Repeat Instability**

A. The haplotype of intermediate alleles (IAs) ascertained from a sample of British Columbia's (B.C.'s) general population (n=49) compared to the Huntington Disease Biobank at the University of British Columbia (UBC-HD BioBank, n=135)

B. The haplotype of general population IAs (n=116) compared to new mutation IAs (n=19) in the UBC-HD Biobank

## 3.4 Discussion

This is the first study to examine the frequency of IAs in a Canadian general population. Our findings suggest that IAs are relatively common among individuals with no known connection to HD. Of persons in the sample of B.C.'s general population, 5.8% had one allele with a CAG size between 27 and 35, inclusive. This genotypic frequency is consistent with other studies that have examined the frequency of IAs in samples not associated with HD, including Portugal's general population (6.0%, n=53/886 individuals) and an ALS patient population (6.4%, n=99/1572 patients) [Ramos *et al.*, 2012; Sequeiros *et al.*, 2010]. Collectively, these studies suggest that 1 in 17 persons in the general population, not associated with HD, have an IA.

The relatively high frequency of IAs in the general population may also shed light on the case reports that suggest an intermediate number of CAG repeats caused the HD phenotype [Andrich *et al.*, 2008; Groen *et al.*, 2010; Ha and Jankovic, 2011; Herishanu *et al.*, 2009; Kenney *et al.*, 2007]. IAs would be expected to occur at the general population rate in samples of patients with various illnesses. Therefore, it would not be surprising to find individuals with an HD phenocopy or HD-like disorder who have a CAG repeat size in the intermediate range simply by chance. In light of this, these case reports do not provide sufficient evidence that the IA actually caused the observed clinical manifestations. In fact, many of these reports have failed to acknowledge the high likelihood of a chance association between IAs and clinical findings. If an IA truly conferred clinical consequences, symptoms would be observed in greater than 6% of individuals with an IA. To our knowledge, no individuals with an IA in the UBC-HD Biobank have displayed clinical manifestations of the disease but research is urgently needed to clarify the clinical consequences of an IA. This research may include prospective studies that examine a large cohort of individuals with an IA for symptoms over time or retrospective case-control studies.

Reduced penetrance HD alleles were also identified in the sample of B.C.'s general population unrelated to HD. Of the individuals examined, 0.4% had one allele with a

repeat size between 36 and 39 CAG. Reduced penetrance HD alleles are not frequently observed among HD alleles [Kremer *et al.*, 1994]. In the Leiden Roster for HD, reduced penetrance alleles were found in only 2.5% of tested persons [Maat-Kievit *et al.*, 2001a; Maat-Kievit *et al.*, 2001b]. Of HD alleles examined at Portugal's diagnostic laboratory, only 3.7% were within the reduced penetrance range [Costa *et al.*, 2003]. In fact, it is estimated that less than 5% of alleles with 36-39 CAG repeats are clinically ascertained given their incomplete penetrance [Falush *et al.*, 2001]. While this is not the first study to identify reduced penetrance HD alleles in a general population, it is the highest frequency of reduced penetrance HD alleles in a population not associated with HD ever reported [Kremer *et al.*, 1994; Sequeiros *et al.*, 2010]. However, as the sample sizes of these studies are relatively small, additional research utilizing larger samples are needed to clarify the true frequency of reduced penetrance alleles in the general population.

While it is possible that these anonymous individuals in the general population with a reduced penetrance HD allele coincidentally belong to a HD family, it is more likely that they represent an unidentified HD mutation, especially given that disease onset due to a reduced penetrance allele would likely be very late, if at all [Falush *et al.*, 2001; McNeil *et al.*, 1997]. Indeed, these reduced penetrance cases may also represent a new mutation for HD due to CAG repeat expansion of an IA. The random ascertainment of these HD alleles in B.C.'s general population may indicate a higher prevalence of HD than previously reported [Warby *et al.*, 2011]. In fact, this finding supports the recent claim that the true prevalence of HD may be underestimated by as much as 80% [Rawlins, 2010; Spinney, 2010]. In the UK, the prevalence of HD is often quoted to be approximately 6 to 7 individuals per 100,000 but based on the number of HD patients receiving care from community organizations, the minimum prevalence is at least 12.4 per 100,000 [Rawlins, 2010]. Studies that aim to revise current HD prevalence estimates in Canada are required. Accurate prevalence estimates will help ensure that appropriate levels of clinical care and support are available for patients and families.

Over half of the IAs identified in B.C.'s general population were found on high-risk haplotypes that predispose to CAG repeat instability. In fact, there was no difference in the proportion of IAs on high-risk haplotypes between IAs ascertained in the general population and those known to have led to a new mutation. This indicates that IAs identified outside known HD pedigrees are susceptible to repeat instability despite no recognized association with HD. Finding a high proportion of IAs on predisposing haplotypes in a general population is consistent with the hypothesis that IAs are the pool from which new mutations are derived [Almqvist *et al.*, 2001; Goldberg *et al.*, 1993b]. Based on this data, the molecular mechanism underlying the occurrence of new mutations may be a step-wise model of CAG repeat instability, where over time, control alleles on haplotypes that predispose to repeat instability undergo multiple expansion events into the IA range and then beyond the pathological threshold [Warby *et al.*, 2009]. In the presence of a predisposing haplotype, the frequency and magnitude of expansion events are modulated by factors known to influence instability, including CAG size, sex of the transmitting parent, and unknown genetic or environmental modifiers.

The majority of general population (72%) and new mutation (63%) IAs in the clinical sample were on haplotypes that confer a high-risk of repeat instability. In fact, there was no statistical difference in the haplotype distribution based on the clinical context of the IA. This supports the claim that the clinical context of an IA is arbitrary. Based on this data, there may be no difference in the propensity of general population and new mutation IAs to undergo repeat expansion given that they are both frequently found on haplotypes that predispose to repeat instability. Therefore, the observed difference in repeat instability previously observed in sperm and transmission studies for new mutation and general population IAs may be an artifact of limited data or may be a consequence of differing CAG size [Chong *et al.*, 1997; Giovannone *et al.*, 1997; Goldberg *et al.*, 1995; Semaka *et al.*, 2010].

The results of this study have important clinical implications. Data presented in Chapter 5 shows that medical genetics service providers do not routinely address

IA-PTR in detail during pre-test genetic counselling and that the familial context of an IA is taken into consideration when assessing the risk of CAG repeat instability. More specifically, genetics professionals tend to be more reassuring about the risk of repeat expansion into the HD range for general population IAs since they have not previously demonstrated instability into the disease range. Based on the relatively high frequency of IAs in the general population, IA-PTRs should be discussed with all clients during their pre-test genetic counselling. Moreover, given that there is no difference in the proportion of high-risk haplotypes for general population and new mutation IAs, the clinical context in which an IAs is identified should not be used in risk assessment for repeat expansion. These clinical implications should be considered for inclusion in an updated version of the HD predictive testing guidelines given that the current version does not address IA-PTRs [IHA and WFN, 1994]. Prior to the revision of the guidelines, however, accurate risk estimates for IA expansion into the HD range are needed. Future studies should also seek to determine the impact of a predisposing haplotypes on the frequency and magnitude of repeat expansion and examine the clinical utility of offering haplotype analysis when an IA is identified as a method for providing more accurate risk estimates for individuals and their families.

# Chapter 4: Significant Risk of New Mutations for Huntington Disease: CAG-Size Specific Risk Estimates of Intermediate Allele Repeat Instability

## 4.1 Synopsis

Risk figures that quantify the likelihood that an IA will expand into the HD range when passed to the next generation are essential to providing accurate genetic counselling. Studies examining germline CAG repeat instability in HD have largely focused on control and HD alleles. There have been only a limited number of studies that have examined IA CAG repeat instability using single sperm analysis. However, the small number of IAs and sperm studied are significant weaknesses of these investigations. These studies have also produced inconsistent rates of instability, particularly expansion into the HD range [Chong *et al.*, 1997; Leeflang *et al.*, 1995]. More specifically, Leeflang and colleagues showed no expansions into the HD range amongst 80 sperm from a single IA with 30 CAG repeats, whereas Chong et al. found the frequency of expansion into the HD range amongst 700 sperm from 4 IAs with CAG sizes of 34 and 35 repeats ranged between 7.5-20.0%. This conflicting data may be a consequence of the small sample size, which may not accurately reflect the true intergenerational mutation rate, or may be due to variability in any of the factors known to influence instability, including CAG size or haplotype [Semaka *et al.*, 2006].

In light of the scarcity of quantified risk estimates for IA repeat instability, it is not surprising that genetic counselling for IA PTR has been described as challenging [Maat-Kievit *et al.*, 2001b; Tassicker *et al.*, 2006]. Moreover, it is not unexpected that individuals who receive an IA-PTR experience confusion and uncertainty about the clinical implications of this result for their children. Large-scale samples are urgently needed to inform clinical practice. Given the correlation of CAG size and incidence of repeat instability observed for HD alleles, there will likely be grades of instability for each CAG size in the intermediate range underscoring the importance of generating CAG size-specific risk estimates. The purpose of this study was to determine CAG-size specific risk estimates for IA repeat instability, including the

frequency and magnitude of contraction and expansion instability, and explore factors that influence this dynamic process, including CAG size, age, and haplotype. Quantified estimates of CAG repeat instability have great clinical relevance and knowledge of factors known to influence instability is important for accurate risk assessment. Risk estimates of expansion into the disease-associated range will also inform individuals' reproductive decision making and may help minimize uncertainty or confusion about the clinical implications of an IA-PTR.

## 4.2 Materials and Methods

### 4.2.1 Recruitment and Donors

Caucasian sperm donors were recruited from medical genetics clinics in Canada, Australia and the Netherlands. Prospective donors were invited to participate in the study by their medical genetics service provider by a mailed letter of invitation, a detailed study information sheet and a consent form (Appendix A.1). Once written informed consent was obtained, donors were mailed a sperm sample collection kit, which included a demographic questionnaire and detailed instructions for sample collection and shipment (Appendix A.1). Sperm samples were collected in the donors' home and shipped either directly to the Centre for Molecular Medicine & Therapeutics (CMMT) or to an intermediary laboratory in Australia or the Netherlands, which collected and stored the samples until they were shipped in bulk to the CMMT. Upon receipt of the samples at the CMMT, all donors, except those from the Netherlands, were sent a letter of thanks and a $50 honorarium (Appendix A.1). Donors were given the option of donating their honorarium to future HD research at the CMMT. Sperm samples stored in the Huntington Disease Biobank at the University of British Columbia (UBD-HD Biobank) were also utilized. Ethical approval was received from all applicable university and hospital ethical review boards.

### 4.2.2 Small-Pool Polymerase Chain Reaction

Southern blot PCR analysis of 'bulk' genomic DNA, composed of thousands of cells,

often >$10^4$ genomic equivalents, has been the traditional method for assessing CAG repeat size and identifies the two constitutional or most common allele sizes. However, this method is highly insensitive in detecting rare mutant alleles, which make up only a small proportion of the sample, that are produced by CAG repeat instability. Samples that contain a high percentage of variant alleles present as a large smear of multiple unresolved alleles on Southern blot analysis [Duyao *et al.*, 1993; Giovannone *et al.*, 1997; Telenius *et al.*, 1995] and do not allow accurate quantification of CAG repeat instability for use in clinical practice. Analysis of single sperm has also been used to examine CAG repeat instability but the technical challenges and considerable financial cost of preparing large-scale single cell samples are considerable limitations [Chong *et al.*, 1997; Leeflang *et al.*, 1999; Leeflang *et al.*, 1995].

Small-pool polymerase chain reaction (SP-PCR) analysis, a highly sensitive methodology, was used to quantitatively assess the frequency and magnitude of germline CAG repeat instability and dissect factors playing a role in this dynamic process [Gomes-Pereira *et al.*, 2004; Jeffreys *et al.*, 1994; Monckton *et al.*, 1995]. SP-PCR provides the opportunity to quantify the degree of CAG repeat instability present in the sperm by detecting not only the presence of common constitutional or progenitor allele CAG sizes, but also those rare variant alleles present in a small subset of cells as a result of CAG repeat instability [Gomes-Pereira *et al.*, 2004; Jeffreys *et al.*, 1994; Monckton *et al.*, 1995]. This methodology also offers the ability to assess large sample sizes at reduced cost. Through serial dilution of bulk genomic DNA into numerous small pools containing only a few genomic equivalents, a larger number of alleles can be analyzed while allowing for the resolution of individual alleles. Amplification of only a few genomic equivalents in each reaction allows low levels of instability to be detected, as rare allele variants are not overwhelmed by the more common progenitor alleles. SP-PCR is thought to increases the probability of amplifying and detecting variant alleles present at levels as low as $\leq$1% [Gomes-Pereira *et al.*, 2004].

#### 4.2.2.1 Differential Lysis and DNA Extraction

Sperm cells were isolated from semen by differential lysis, which separates sperm cells from other 'round' cells of somatic origins, including epithelial cells and leucocytes, given their resistance to lysis by sodium dodecyl sulfate (SDS) [Gomes-Pereira *et al.*, 2004; Jeffreys *et al.*, 1994; Monckton *et al.*, 1995]. While the proportion of round cells in semen varies based on the individual, it is estimated that ≥5% of the cells in semen are somatic. Inclusion of somatic cells in the analysis would skew the calculated germline instability estimates as there are thought to be differences between somatic and germline instability in HD [Swami *et al.*, 2009]. Using differential lysis, haploid genomic DNA was extracted from isolated sperm cells eliminating a major contribution of diploid DNA from somatic cells. Somatic cell lysates were discarded.

Round cells from 500uL semen samples were lysed and separated from sperm cells by three successive washes with 1mL 1% SDS in 1xSSC, followed by one wash each in 1mL 1xSSC and 1mL 0.5xSSC. After centrifugation, sperm pellets were then resuspended and incubated for 2-4 hours in 200uL buffered 4% SDS (20mM Tris-Cl pH 8.0, 20mM EDTA, 200mM NaCl) with 80mM dithiothreitol (DTT) and 2.5uL Qiagen Proteinase K Solution [Hilden, Germany] to promote sperm cell lysis. Following sperm lysis, genomic sperm DNA was extracted by silica column purification with the Qiagen DNeasy Blood and Tissue Kit according to the manufacturer's instructions. 200uL sperm lysis volume was mixed with 200uL 96% EtOH and 200uL Qiagen Buffer AL immediately prior to column application. Sperm DNA was eluted in 100uL Qiagen TE Buffer (10 mM Tris-Cl pH 8.0, 1 mM EDTA). Genomic sperm DNA was digested with *Hin*dIII [New England Biolabs, Ipswich, MA] and requantified with a parallel blank *Hin*dIII digest prior to dilution for small-pool PCR.

#### 4.2.2.2 Serial Dilution

*Hin*dIII-digested sperm DNA was quantified by UV spectroscopy on Nanodrop ND-

1000 spectrophotometer [ThermoFisher Scientific, Waltham, MA] by A260/A230 ratio and serially diluted to 60 pg/uL working concentration immediately prior to the SP-PCR assay. 7.5pg of digested sperm DNA was added to each SP-PCR reaction, amplifying an average of 1.1 diploid genomic equivalents (2.2 haploid genomic allelic equivalents) per reaction. Following a Poisson distribution, at this DNA concentration approximately 10% of the SP-PCR reactions were expected to contain no genomic equivalents and thus would fail to amplify a product.

#### 4.2.2.3 Polymerase Chain Reaction

A sensitive hemi-nested SP-PCR assay was optimized that allowed the resolution of the CAG repeat tract from individual sperm cells. The SP-PCR assay did not amplify the proline (CCG) tract adjacent to the CAG repeat. A first round PCR was carried out in a 5uL reaction volume containing the primers HD344F_HEX (5'-HEX-CCTTCGAGTCCCTCAAGTCCTTC-3', 0.6 mM) and HD482R (5'-GGCTGAGGAAGCTGAGGAG-3', 0.6 mM), using a custom buffer mix designed to assist amplification of the GC-rich repeat region (1X PCR Buffer (10mM Tris-HCl, 50mM KCl, 1.5mM MgCl2, pH 8.3) [Roche, Mannheim, Germany], 3.5% HiDi Formamide [Applied Biosystems, Foster City, CA], 15% glycerol with 0.2 mM each dNTP [Invitrogen, Carlsbad, CA] and 0.25 U Roche GMP Grade *Taq* DNA Polymerase [Roche]. A volume of 60pg/uL digested sperm DNA was included in each master mix preparation such that 7.5 pg was present in each 5uL reaction when distributed over eight 96-well plates. A parallel blank master mix, without diluted DNA, was prepared for addition of eight negative controls distributed across each plate of SP-PCR reactions. PCR conditions consisted of an initial denaturation step of 3 min at 95C, followed by 15 cycles of 95°C, 61°C, and 72°C for 1 minute each, with a terminal elongation step of 5 min at 72°C.

PCR products from the first round reaction were diluted 1/10 in DNAse-, RNAse-free dH2O and 2 ml of each dilution used as template for a 25uL second round reaction. The second round reaction mix was identical to that of the first round, except for

heminested modified primers HD344F_HEX (5'-HEX-CCTTCGAGTCCCTCAAGTCCTTC-3', 0.6 mM) and HD450R_PT (5'-GTTTGGCGGCGGTGGCGGCTGTTG-3', 0.6 mM). Second round cycling conditions were identical to those of the first round, except 33 cycles were performed.

There is a significant risk of contamination in SP-PCR due to the amplification of a very small number of genomic equivalents per reaction and the possibility of amplifying extraneous DNA. Therefore, all SP-PCR reactions were set-up in a bleached laminar flow hood using careful aseptic technique and laboratory equipment (i.e. pipettes, tips, etc) and reagents (i.e. primers, buffers, etc), which were specifically designated for SP-PCR.

The forward primer in the second round amplification was fluorescently labeled with HEX [Invitrogen], allowing fragment analysis of PCR products and accurate measurement of CAG repeat length using an automated ABI 3730XL sequencer and GeneMapper v.4.0 software with GS 500 LIZ internal size standard [Applied Biosystems]. Eight DNA-negative reactions were included on each 96 well plate, and similarly diluted and transferred to second round reactions, to control for contamination. Each batch of four SP-PCR plates contained second round CAG sizing reactions performed with identical second round master mix of six positive controls of known CAG size and two duplicate genotyping reactions of donor genomic sperm DNA.

#### 4.2.2.4 Reconstruction Experiments

Reconstruction experiments were conducted to assess our ability to detect different levels of instability. Specifically, two somatic (blood) DNA samples with a 17/30 CAG and 20/32 CAG genotype were mixed at different ratios, including 1:0, 1:1, 2:1, 10:1, and 50:1, and co-amplified. We were able to reliably detect the four different alleles at the expected frequencies based on the ratio of the mixture. For example, when

the samples were mixed at a 10:1 ratio, on average, for every ten 30 CAG alleles, we detected one 32 CAG allele. We also did not observe any difference in our ability to detect varying amounts of instability between the lower and upper allele. These experiments suggest that the SP-PCR methodology could reliably detect different levels of instability.

#### 4.2.2.5 Quantification of the Number of Sperm Examined

Approximately eight 96-well plates of SP-PCR reactions were analyzed per sperm donor. Excluding the positive and negative controls included on each plate, this equals an average of 688 SP-PCR reactions per donor. Based on the input of approximately 2.2 haploid genomic equivalents per SP-PCR reaction and a failure rate of roughly 10%, an average of 1200 alleles were examined for each donor. This equates to about 600 equivalents each of the donor's lower and upper allele.

While the number of haploid genomic equivalents in each SP-PCR reaction was theoretically known, the technical challenges associated with determining bulk DNA concentrations and transferring small amounts of DNA made it necessary to calculate the number of input DNA molecules empirically. Empirical calculations were important because while 2.2 haploid genomic equivalents were theoretically added to each reaction, some reactions may contain 0, 1, 2, and less frequently 3 or 4 allelic equivalents. Precisely determining the number of DNA molecules examined was critical to determining accurate CAG repeat instability estimates.

The number of SP-PCR reactions that failed to produce a product was used to empirically calculate the average number of input haploid DNA molecules per SP-PCR reaction based on the Poisson distribution. For each allele, using the ratio of negative reactions (i.e. no PCR product detected) to total number of reactions analyzed, designated *f(0)*, the average number of input molecules (*m*) amplified in each small pool reaction was determined. Based on the empiric calculation of the average number of input molecules per SP-PCR reaction, the total number of

molecules examined was determined.

Quantification of the number of sperm examined for each allele, utilized the following formulas derived from the Poisson distribution [Gomes-Pereira *et al.*, 2004; Monckton *et al.*, 1995]:

1. ***f(0) = total # SP-PCR reactions with no product / total # SP-PCR reactions***
2. ***m = -ln f(0)***
3. ***Total # molecules examined = m * total # SP-PCR reactions***

The total number of lower and upper allele molecules examined was calculated separately. For example, when studying the upper allele, a reaction was counted as having a product only if the upper progenitor size or a variant thereof was observed. In other words, reactions with only the lower progenitor allele or a variant thereof were scored as a reaction with no product from the upper allele. Determining the origin of a variant allele as from either the lower or upper progenitor allele was based on thresholds that were set using the largest expansion observed for control alleles. More specifically, nine control samples with a normal genotype (i.e. 17/17 CAG, 17/19 CAG) from the UBC-HD Biobank were studied to determine the magnitude of control allele expansions. Based on the CAG size of the largest expansion observed, thresholds were set that allowed the separation of the donor's lower and upper allele and establish which progenitor allele a particular variant was from (i.e. the variant is an expansion of the donor's lower allele or a contraction of their upper allele). For example, the largest expanded variant observed for control alleles with 17 CAG repeats was 19 CAG, thus the upper expansion threshold for a 17 CAG allele was set at 20 CAG repeats, inclusive. The CAG size distribution of alleles from control donors ($\leq$26 CAG, n=9) and the control allele expansions thresholds are reported in Figure 4.1 and Table 4.1.

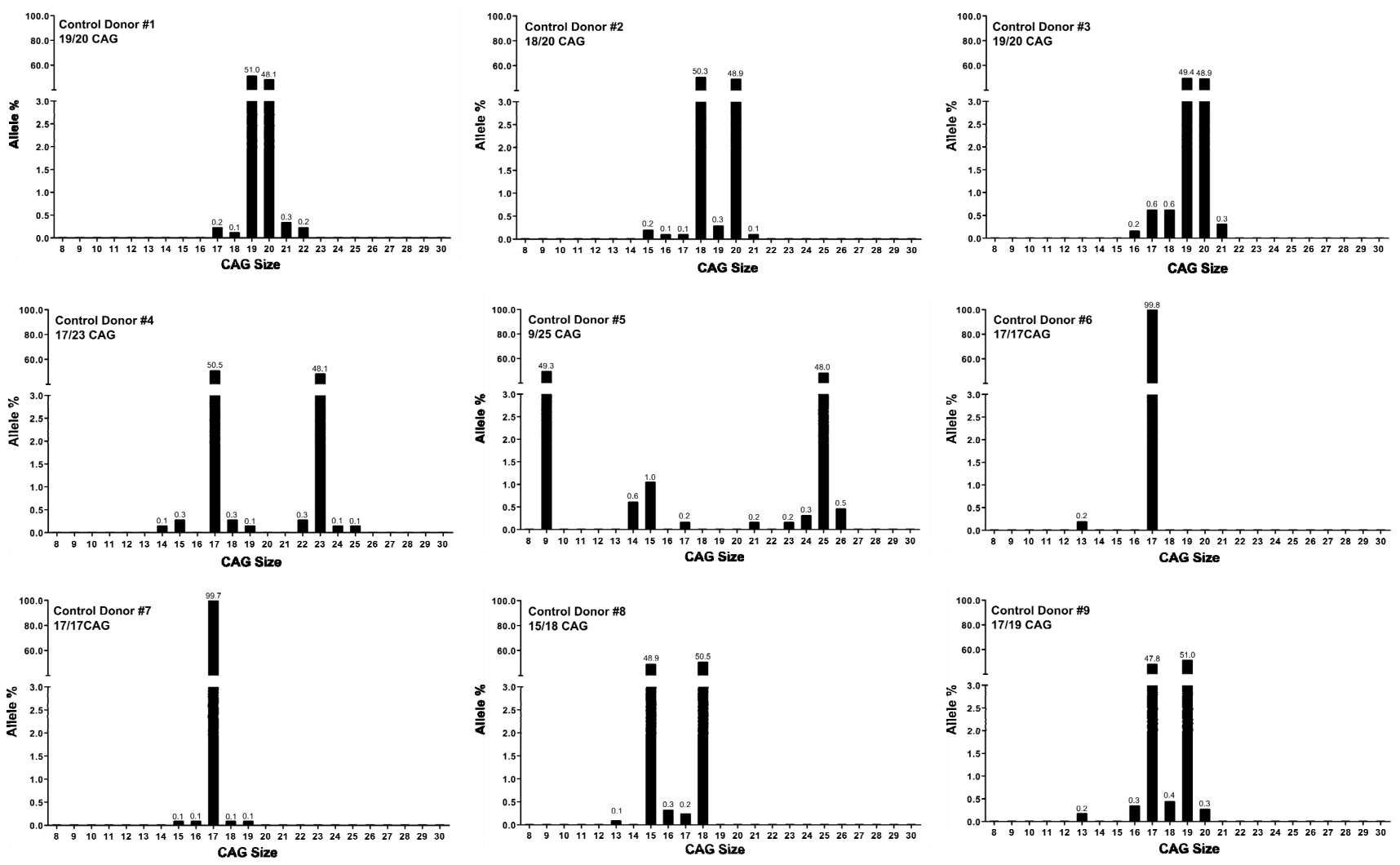



**Figure 4.1 CAG Size Distribution of Alleles from Control Donors**

| CAG Size | Largest CAG Observed | Relative CAG Expansion Threshold |
|---|---|---|
| **15** | 18 | 18 |
| **16** | -- | 19 |
| **17*** | 19 | 20 |
| **18** | 21 | 21 |
| **19** | 22 | 22 |
| **20** | 22 | 23 |
| **21** | -- | 24 |
| **22** | -- | 25 |
| **23** | 25 | 26 |
| **24** | -- | 27 |
| **25** | 26 | 28 |
| **26** | -- | 29 |

-- CAG size not examined

* Based on two donors with homozygous genotype

**Table 4.1 Relative CAG Expansion Thresholds for Control Alleles**

The relative CAG expansion thresholds for control alleles (≤26 CAG) were based on the largest CAG size observed in sperm samples from donors with control genotypes. These expansions thresholds were used to determine the origin of a variant allele as from either the lower or upper progenitor allele.

#### 4.2.2.6 GeneScan Analysis

GeneScan chromatographs of each SP-PCR reaction were manually scored for the presence of progenitor and variant alleles. PCR amplification of a single stable trinucleotide repeat allele commonly results in a stutter pattern on GeneScan analysis, which consists of a large peak of high intensity, trailed by 2-4 peaks of lower intensity [Coolbaugh-Murphy *et al.*, 2005; Macdonald *et al.*, 2011], Figure 4.2.A). A threshold of minimum peak intensity, 300 relative fluorescent units, was utilized when scoring alleles and peaks with intensities below this threshold were excluded [Macdonald *et al.*, 2011]. The peak heights of alleles and their stutter

varied amongst the reactions, but in most cases the actual allele had a higher peak intensity than the stutter peaks. If two or more alleles are present in a single reaction and differ in size by one CAG repeat, the peak of the smaller allele will display the highest peak intensity, with its peak area being greater than 150% of the larger allele's peak (Figure 4.2.B & C). If the alleles present in a reaction differ in size by two or more CAG repeats, two distinct peaks with high intensity, each followed by stutter peaks, will be observed (Figure 4.2.D, E, & F). Approximately 10% of the SP-PCR reactions yielded no product due to lack of input DNA (Figure 4.2.G).

CAG sizing of progenitor and variant alleles was determined relative to positive controls of known CAG size. Approximately 90 positive control samples, with a range of different CAG sizes, including control ($\leq$26 CAG), intermediate (27-35 CAG) and HD ($\geq$36 CAG) repeat lengths were used to establish CAG sizing bins in the GeneMapper software. All PCR products were sized using the sizing bins. Moreover, the eight positive control samples, included on each batch of four 96-well plates, acted as an internal size standard by validating the accuracy of the CAG size bins.

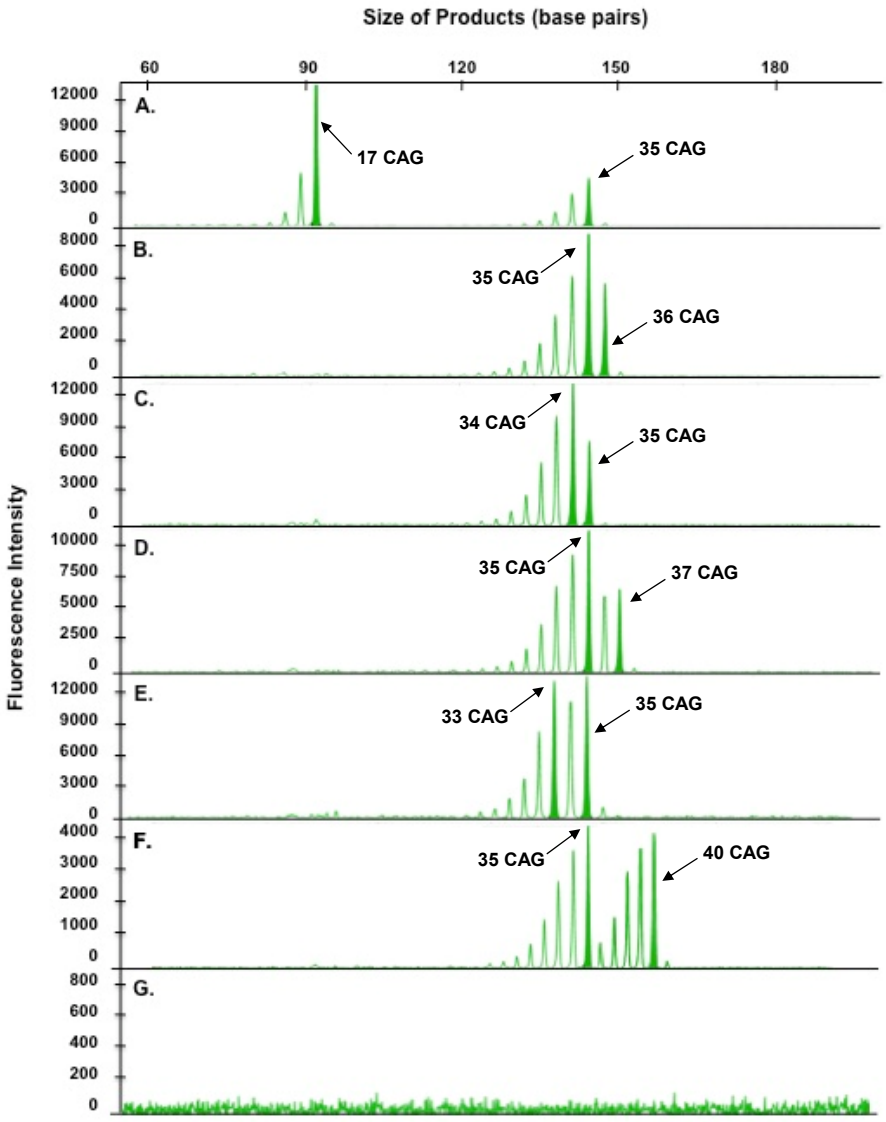



**Figure 4.2 GeneScan Chromatograms of Small-Pool PCR Products**

Filled green peaks represent either progenitor or mutant alleles. Unfilled peaks represent stutter peaks, which are artifacts of PCR amplification. A. Two progenitor allele peaks of 17 and 35 CAG trailed by a series of smaller stutter peaks. B. A progenitor allele peak of 35 CAG and a mutant allele peak of +1 CAG repeat expansion (36 CAG). The area of the progenitor allele peak was at least 150% greater than the area of the +1 CAG mutant allele peak. C. A progenitor allele peak of 35 CAG and a mutant allele peak of -1 CAG repeat contraction (34 CAG). The area of the -1 CAG mutant allele peak was at least 150% greater than the area of the progenitor allele peak. D. The progenitor allele peak of 35 CAG and a mutant allele peak of +2 CAG repeat expansion (37 CAG). E. The progenitor allele peak of 35 CAG and a mutant allele peak of -2 CAG repeat expansion (33 CAG). F. The progenitor allele peak of 35 CAG and a mutant allele peak of +5 CAG repeat expansion (40 CAG). G. Failed spPCR reaction due to lack of input DNA.

### 4.2.3 Haplotype Analysis

Haplotype analysis of the sperm donors was based on the study by Warby et al. [2009] and described in detail in Chapter 3 Material and Methods (page 68). Briefly, genomic sperm DNA from each sample was genotyped on a customized Illumina GoldenGate Assay [Illumina, San Diego, CA] at each of 96 SNPs across the HTT gene region, including 22 tagging SNPs (tSNPs) used to define three major haplogroups (A, B and C) and 5 haplogroup A variants (A1, A2, A3, A4 and A5). Haplotypes were phased to CAG size using either pedigree trios, a known haplogroup association with the polymorphic CCG repeat tract adjacent to the CAG repeat, or haplotype homozygosity. We were unable to phase a small proportion of donors (n=5) using any of these methods.

For the purpose of this study, haplotype A variants 1 and 2 conferred the highest risk for having a CAG-expanded allele and, thus, are collectively referred to as *high-risk* haplotypes for repeat instability. All other haplogroup A variants and major haplogroups (i.e. B, C, A3, A4, A5, O), which did not confer a high likelihood of a CAG expansion, are collectively referred to as *low-risk* haplotypes.

### 4.2.4 Calculating CAG-Size Specific Instability Estimates

Germline CAG repeat instability refers to changes in repeat length, including increases and decreases in repeat size, from the constitutional or progenitor allele size, upon transmission to the next generation. In each sperm sample, the two most frequent alleles represented the two progenitor alleles and matched the donor's genotyping results. Notably, however, as HD alleles ($\geq$36 CAG) demonstrated significant CAG repeat instability, the most frequent CAG size in the sperm sample did not always match the donor's genotype and represent the two progenitor alleles.

The frequency of CAG repeat instability was defined as the proportion of variant alleles that differed in repeat length from the respective progenitor allele size and was calculated using the following formula for the lower and upper allele independently:

***Frequency of repeat instability = total # variants observed / total # sperm examined***

Following the same principle, additional instability estimates were calculated including the frequency of contraction instability and expansion instability.

### 4.2.5 Limitations of Small-Pool Polymerase Chain Reaction

It is well known that PCR can favor the amplification of smaller repeat tracts [Jeffreys *et al.*, 1988]. In this study, the average number of lower allele examined per donor was slightly higher (641 molecules) than the number of upper alleles examined (604 molecules, p=0.002). Thus, it is possible that smaller alleles had a competitive advantage when present in reactions that also contained a larger allele. This may explain the slighter higher amplification of lower alleles and suggests that there may be a small bias towards the amplification of contracted variants compared expanded variants. Consequently, the frequency of contraction instability may be slightly overestimated, whereas the frequency of expansion instability may be somewhat underestimated [Crawford *et al.*, 2000; Leeflang *et al.*, 1995; Macdonald *et al.*, 2011]. Therefore, these estimates must be interpreted as relative, instead of precise, instability rates.

There are concerns that variants detected by SP-PCR could be PCR artifacts rather than true variant alleles [Crawford *et al.*, 2000; Gao *et al.*, 2008; Macdonald *et al.*, 2011]. Consequently, if PCR artifacts were mistaken for variant alleles, it is possible that the instability estimates are slightly overestimated. However, SP-PCR methodology studies indicated that artifacts are uncommon [Coolbaugh-Murphy *et al.*, 2004]. Moreover, there are some key pieces of evidence that indicate artifacts were rare in the current study and likely do not significantly impact the accuracy of the instability estimate produced. Firstly, all alleles scored were discrete peaks with similar intensities that conformed to the expected stutter pattern. Secondly, alleles were never more frequent than expected based on the Poisson distribution; in other words, the average number of alleles in each SP-PCR conformed to the expected 2.2 haploid genomic equivalents. Lastly, all the DNA-negative control reactions were

clean and the distribution pattern of variant allele differs from sample to sample.

### 4.2.6 Statistical Analysis

Statistical analysis was largely descriptive in nature. Fisher's exact test or Chi-square analysis was used to examine differences in the frequency of instability for IAs on low and high-risk haplotypes using GraphPad Prism Version 5.0A (GraphPad Software, San Diego California USA). Differences in mean CAG size were assessed using Student's t-test. Multiple linear regression analysis was performed to dissect factors influencing CAG repeat instability. The response (dependent) variable was the frequency of CAG repeat instability and the explanatory (independent) variables were CAG size, donor age, and haplotype (low or high-risk). As, linear regression requires the dependent variable to be linear, a 'log normal' transformation of the frequency of CAG repeat instability was performed prior to performing the regression analysis.

## 4.3 Results

### 4.3.1 Sample Size

Thirty-one semen samples were received from Caucasian males with an intermediate or HD allele at the Canadian (n=8), Australian (n=5), and Dutch (n=18) medical genetics clinics. Six samples were excluded from the analysis - one sample contained no DNA, likely the donor was azoospermic, and five samples had a double IA genotype (i.e.27/29 CAG), which precluded accurate separation of the donor's upper and lower allele variants. Ten semen samples stored in UBC-HD Biobank were also examined.

Thirty-five semen samples were analyzed, for a total of 70 alleles, which ranged in CAG size from 15 to 42 CAG. There were 35 control ($\leq$26 CAG), 31 intermediate (27-35 CAG), and 4 HD ($\geq$36 CAG) alleles. A total of 43580 sperm cells were examined – 22446 control, 18763 intermediate, and 2371 HD sperm cells. The number of alleles and sperm examined at each CAG size is reported in Table 4.2.

### 4.3.2 Relationship Between CAG Size and Repeat Instability

There was a significant non-linear relationship between CAG size and repeat instability, where the frequency of CAG repeat instability increased with increasing CAG size (Pearson's correlation = 0.794, n=70, p<0.001, Figure 4.3). A significant correlation between CAG size and the frequency of repeat contraction (r=0.753, n=70, p<0.001, Figure 4.3) and expansion (r=0.703, n=70, p<0.001, Figure 4.3) was also observed. However, there was a more marked increase in the frequency of repeat expansion compared to contraction, with expansion dramatically increasing with increasing CAG size.

Scatter plots of the frequency of instability based on CAG size highlighted two outliers. The first, a 31 CAG allele had a higher rate of repeat contraction and expansion, resulting in an overall increase in the frequency of instability. The second, a 39 CAG allele had an increased frequency of contraction and decreased rate of repeat expansion. These outliers were excluded from all subsequent analyses so as not to bias the CAG-size specific estimates generated. These outliers have been sent for sequence analysis to explore whether cis-acting genetic factors, such as point mutations in the 12 base pair sequence between the CAG and CCG tract, may explain their atypical instability.

### 4.3.3 Frequency of CAG Repeat Instability

Control alleles (n=35) were relatively stable, with only 2.2% (n=490/22446 sperm) demonstrating CAG repeat instability (Table 4.3). Over the control CAG size range there was a 5-fold increase in the frequency of instability, with 15 CAG alleles (n=2) showing 1.0% (n=13/1309 sperm) instability and 25 CAG alleles (n=1) having 5.4% (n=42/788) instability. Of unstable control alleles, the proportion of contractions (74.1%, n=363/490 sperm) was greater than expansions (25.9%, n=127/490). The overall frequency of repeat contraction (1.6%, n=363/22446 sperm) was also greater than expansion (0.6%, n=127/22446). While both the frequency of repeat contraction and expansion increased with increasing CAG size, there was a marked increase in

repeat contraction compared to expansion. Contraction instability increased 7-fold over the control CAG range while expansion increased only 3-fold. More specifically, repeat contraction ranged from 0.6% (n=8/1309 sperm) to 4.1% (n=32/788) for 15 and 25 CAG alleles, respectively, whereas expansion instability varied from 0.4% (n=5/1309 sperm) to 1.3% (n=10/788). Generally, control alleles did not expand beyond the upper limit of the control CAG size range (>26 CAG) into another size range associated with different clinical implications. However, 0.5% (n=4/788) of 25 CAG alleles expanded into the intermediate CAG size range (Table 4.5).

Collectively, 15.8% (n=2869/18198 sperm) of IAs (n=30) were unstable (Table 4.3). There was a 6-fold increase in repeat instability over the intermediate CAG size range, with 27 CAG alleles (n=5) demonstrating 5.5% (n=161/2907 sperm) repeat instability and 35 CAG alleles (n=4) having 33.0% (n=756/2290) instability. Of unstable IAs, the proportion of contractions (50.8%, n=1459/2869 sperm) and expansions (49.2%, n=1412/2869) were relatively equal. While the frequency of repeat contractions (8.0%, n=1457/18198 sperm) and expansions (7.8%, n=1412/18198) were equivalent for IAs as a group, expansions did not actually exceed contractions until >33 CAG. Within the intermediate CAG size range, the increase in repeat expansion was more striking than contraction. There was a 10.5-fold increase in expansion instability, compared to a 3.5-fold increase in repeat contraction. From 27 to 35 CAG, the frequency of repeat expansion ranged from 2.0% (n=58/2907 sperm) to 21.0% (n=481/2290), whereas contraction instability extended from 3.5% (n=103/2907) to 12.0% (n=275/2290).

Of IAs that contracted in CAG size, 87.4% (n=1273/1457 sperm) of the contractions were within the intermediate CAG size range, only 12.6% (n=184/1457) contracted into the control range. The frequency of repeat contractions into the control CAG size range was 1.0% (n=184/18198 sperm) (Table 4.4). Collectively, 3.4% (n=610/18198 sperm) of IAs expanded into the HD range resulting in a new mutation (Table 4.5). The new mutation rate of IAs ranged from 0.1% (n=4/2907 sperm) to 21.0% (n=481/2290) for 27 and 35 CAG alleles, respectively, which equals a 200-

fold increase over the IA CAG size range. The largest increase in the frequency of expansion into the HD range occurred between 34 and 35 CAG, where there was a 9-fold increase in HD expansions. Between 33 and 34 CAG, there was a 2.5-fold increase in new mutation expansions. Of expansions that crossed the disease threshold, 92.6% (n=565/610 sperm) were within the reduced penetrance CAG size range compared to 7.4% (n=45/610) in the full penetrance range.

While only 3 HD alleles (39, 41, 42 CAG) were examined, they were exceedingly unstable, with an instability rate of 74.1% (n=1344/1813 sperm, Table 4.3). Of unstable HD alleles, the proportion of expansions (79.9%, n=1074/1344 sperm) was greater than contractions (19.1%, n=270/1344). The overall frequency of repeat expansion (59.2%, n=1074/1813 sperm) was also greater than contractions (14.9%, n=270/1813). A small proportion of HD alleles reverted to control (0.1%, n=2/1813 sperm) or intermediate (0.9%, n=16/1813) alleles (Table 4.4). Given the CAG size of the HD alleles examined, all expansions were into/within the full penetrance CAG size range (Table 4.5).

### 4.3.4 Magnitude of CAG Repeat Instability

The magnitude of CAG repeat instability was quantified by the repeat length variation between the progenitor and variant allele CAG sizes (i.e. +1 CAG, +10 CAG, -5 CAG, -15 CAG). The magnitude of contraction and expansion instability increased with increasing CAG size (Figure 4.4). For control alleles (n=35), the repeat length variation of contractions was greater than expansions (Figure 4.4). More specifically, the largest repeat length variation observed for contractions was -10 CAG compared to +3 CAG for expansions. Approximately 0.5% (n=116/22446 sperm) of control alleles contracted by one CAG repeat, whereas 0.4% (n=91/22446) expanded by one repeat (Figure 4.5.A). Moreover, 0.1% (n=22/22446 sperm) of control alleles contracted by greater than 5 CAG repeats but no repeat length variation greater than 5 CAG repeats was observed for expanded control alleles.

For IAs, the magnitude of repeat instability was greater for expansions compared to contractions (Figure 4.4). In particular, the largest repeat length variation observed for contractions was -13 CAG whereas the largest variation for expansions was +20 CAG. Approximately 5.0% (n=907/18198 sperm) of IAs contracted by one CAG repeat, 1.8% (n=327/18198) by two repeats, and 0.5% (n=88/18198) by three repeats (Figure 4.5.B). Conversely, 6.0% (n=1089/18198 sperm) of IAs expanded by one CAG repeat, 1.1% (n=197/18198) by two repeats, and 0.2% (n=42/18198) by three repeats. The percentage of alleles with repeat length variations beyond 5 CAG repeats was similar between contractions (0.3%, n=57/18198 sperm) and expansions (0.3%, n=62/18198)

While only three HD alleles were examined, the magnitude of expansion instability appears to be greater than contractions (Figure 4.4). Specifically, the largest repeat length variation observed for expansions was +16 CAG, whereas -12 CAG was the largest variation for contractions. Approximately 7.9% (n=143/1813 sperm) of HD alleles contracted by one CAG repeat, 3.9% (n=70/1813) by two repeats, and 1.6% (n=70/1813) by three repeats (Figure 4.5.C). For expanded HD alleles, 19.4% (n=352/1813 sperm) increased by one CAG repeat, 13.4% (n=243/1813) by two repeats, and 9.4% (n=243/1813) by three repeats. The frequency of HD alleles that expanded (7.9%, n=143/1813 sperm) beyond 5 repeats was greater than contractions (0.6%, n=11/1813 sperm).

### 4.3.5 Impact of Haplotype on CAG Repeat Instability

Haplotype data was available for 60 alleles, including 31 control, 26 intermediate, and 3 HD alleles (Table 4.6). In order to assess the impact of haplotype on the frequency and magnitude of repeat instability, the influence of CAG size on instability must be controlled for. The mean CAG size of IAs on low-risk (n=8, 30.3 CAG) and high-risk (n=18, 31.5 CAG) haplotypes was not significantly different (p=0.3183). However, the mean CAG size of control alleles significantly differed between low-risk (n=24, 17.4 CAG) and high-risk (n=7, 21.0 CAG, p=0.0002) haplotypes. Thus, control alleles found on high-risk haplotypes are associated with

an increased CAG size. The small sample size of HD alleles (n=3) precluded statistical comparison.

IAs on high-risk haplotypes demonstrated greater frequency of instability (18.3%,n=2070/11322) compared to those alleles on low-risk haplotypes (10.2%, n=467/4580, p<0.0001, Figure 4.6.B). However, haplotype did not influence the proportion of IAs contractions and expansions (p=0.8730). IAs on high-risk haplotypes also had a greater magnitude of repeat instability, with the repeat length variation ranging from +17 to -13 CAG repeats, compared to alleles on low-risk haplotypes, which ranged from +12 CAG to -8 CAG (Figure 4.7). While the impact of CAG size cannot be eliminated, a similar relationship was observed for control alleles, where alleles on high-risk haplotypes (18.3%, n=151/4819) had greater instability than low-risk haplotypes (10.2%, n=281/15109, p<0.0001, Figure 4.6.A). There was no difference in the proportion of control alleles that contracted or expanded based on haplotype (p=0.8730). Unlike IAs, the haplotype of control alleles did not appear to significantly impact the magnitude of repeat instability (Figure 4.7).

### 4.3.6 Factors Influencing CAG Repeat Instability

Multiple linear regression analysis was performed to dissect factors influencing CAG repeat instability. The response (dependent) variable was the frequency of CAG repeat instability and the explanatory (independent) variables were CAG size, donor age, and haplotype (high- or low-risk). The regression model was highly significant (p<0.0001) with CAG size, age, and haplotype explaining 88.6% of the variance ($R^2$=0.886) in the frequency of CAG repeat instability. While CAG size (p<0.0001), age (p=0.039), and haplotype (p<0.0001) were all significantly correlated with the frequency of repeat instability, CAG size (p<0.0001) and age (p=0.006) were the only significant predictors of repeat instability. CAG size (Beta coefficient=0.908) was 7-fold better at predicting instability than age (Beta coefficient=0.129). Haplotype (p=0.611) was not found to be a significant predictor of instability.

| CAG Size | Number of Alleles | Number of Sperm |
|---|---|---|
| **Total:** | 70 | 43580 |
| **15** | 2 | 1309 |
| **16** | 1 | 469 |
| **17** | 18 | 11733 |
| **18** | 3 | 1752 |
| **19** | 2 | 1031 |
| **20** | 4 | 2684 |
| **22** | 3 | 1827 |
| **24** | 1 | 853 |
| **25** | 1 | 788 |
| **Control Total:** | 35 | 22446 |
| **27** | 5 | 2907 |
| **28** | 3 | 1695 |
| **29** | 3 | 1670 |
| **30** | 4 | 2337 |
| **31** | 3 | 1862 |
| **32** | 1 | 561 |
| **33** | 2 | 1591 |
| **34** | 6 | 3850 |
| **35** | 4 | 2290 |
| **Intermediate Total:** | 31 | 18763 |
| **39** | 2 | 1347 |
| **41** | 1 | 597 |
| **42** | 1 | 427 |
| **HD Total:** | 4 | 2371 |

**Table 4.2 Summary of the Number of Control, Intermediate, and Huntington Disease Alleles and Sperm Examined from 35 Donors**

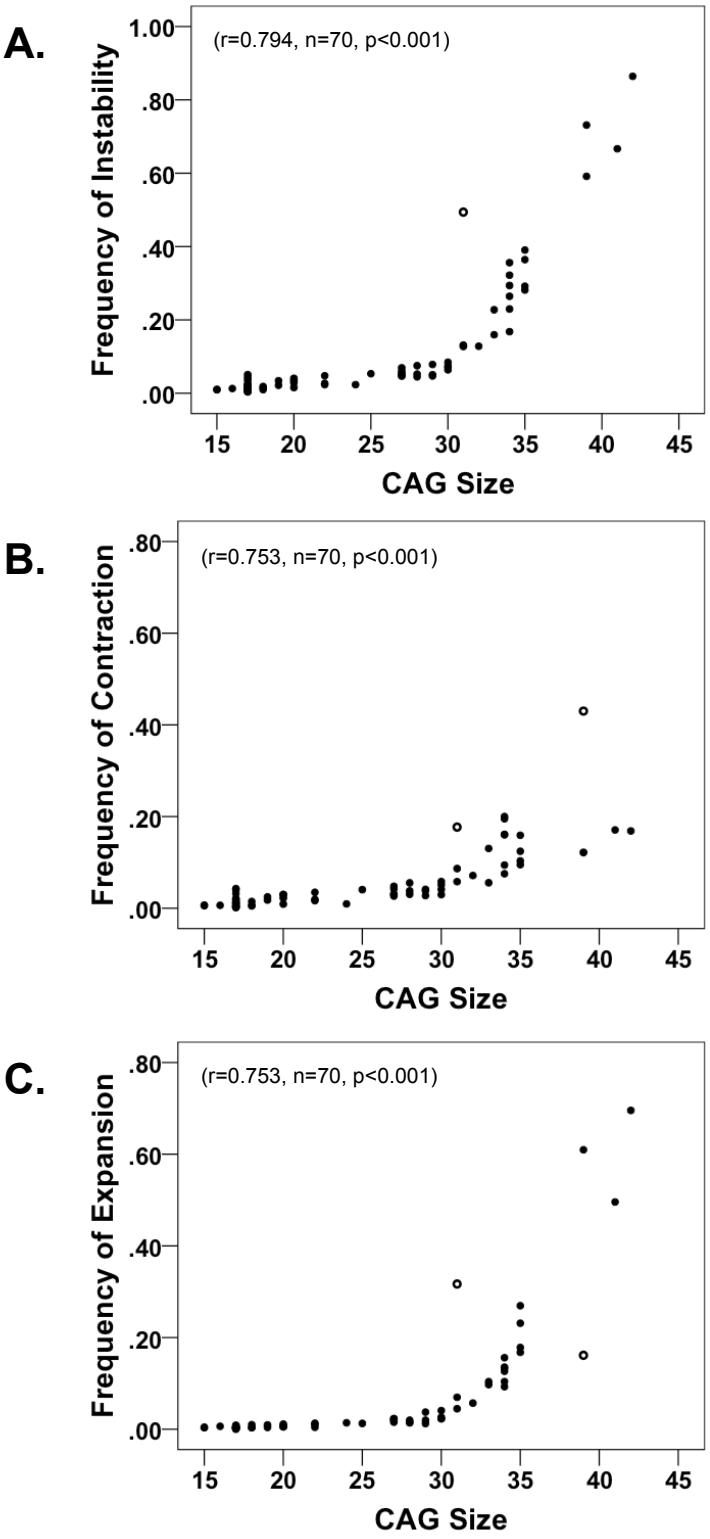



**Figure 4.3 Nonlinear Relationship Between CAG Size and the Frequency of Repeat Instability**
A. Total repeat instability B. Contraction repeat instability C. Expansion repeat instability. Unfilled circles identify two outliers, a 31 CAG & 39 CAG allele, which were removed from subsequent analyses

| CAG Size | Frequency of CAG Repeat Instability | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Stable | | Unstable | | Contraction | | Expansion | |
| | n | % | n | % | n | % | n | % |
| **15** | 1296 | 99.0 | 13 | 1.0 | 8 | 0.6 | 5 | 0.4 |
| **16** | 463 | 98.8 | 6 | 1.2 | 3 | 0.6 | 3 | 0.6 |
| **17** | 11517 | 98.1 | 216 | 1.9 | 173 | 1.5 | 43 | 0.4 |
| **18** | 1727 | 98.5 | 25 | 1.5 | 15 | 0.9 | 10 | 0.6 |
| **19** | 1002 | 97.2 | 29 | 2.8 | 22 | 2.1 | 7 | 0.7 |
| **20** | 2607 | 97.1 | 77 | 2.9 | 58 | 2.2 | 19 | 0.7 |
| **22** | 1765 | 96.6 | 62 | 3.4 | 44 | 2.4 | 18 | 1.0 |
| **24** | 833 | 97.7 | 20 | 2.3 | 8 | 0.9 | 12 | 1.4 |
| **25** | 746 | 94.6 | 42 | 5.4 | 32 | 4.1 | 10 | 1.3 |
| **Control:** | **21956** | **97.8** | **490** | **2.2** | **363** | **1.6** | **127** | **0.6** |
| **27** | 2746 | 94.5 | 161 | 5.5 | 103 | 3.5 | 58 | 2.0 |
| **28** | 1599 | 94.3 | 96 | 5.7 | 69 | 4.1 | 27 | 1.6 |
| **29** | 1573 | 94.2 | 97 | 5.8 | 60 | 3.6 | 37 | 2.2 |
| **30** | 2167 | 92.7 | 170 | 7.3 | 105 | 4.5 | 65 | 2.8 |
| **31** | 1129 | 87.0 | 168 | 13.0 | 95 | 7.3 | 73 | 5.6 |
| **32** | 489 | 87.2 | 72 | 12.8 | 40 | 7.1 | 32 | 5.7 |
| **33** | 1284 | 80.7 | 307 | 19.3 | 147 | 9.2 | 160 | 10.1 |
| **34** | 2808 | 73.0 | 1042 | 27.0 | 563 | 14.6 | 479 | 12.4 |
| **35** | 1534 | 67.0 | 756 | 33.0 | 275 | 12.0 | 481 | 21.0 |
| **Intermediate:** | **15329** | **84.2** | **2869** | **15.8** | **1457** | **8.0** | **1412** | **7.8** |
| **39** | 212 | 26.8 | 577 | 73.2 | 96 | 12.2 | 481 | 61.0 |
| **41** | 199 | 33.3 | 398 | 66.7 | 102 | 17.1 | 296 | 49.6 |
| **42** | 58 | 13.5 | 369 | 86.5 | 72 | 16.9 | 297 | 69.6 |
| **HD:** | **469** | **25.9** | **1344** | **74.1** | **270** | **14.9** | **1074** | **59.2** |

**Table 4.3 CAG-Size Specific Risk Estimates for Repeat Instability**
The percentage of stable, unstable, contracted, and expanded sperm is report as a percentage of the total number of sperm examined per CAG size as reported in Table 4.2. Two outliers were excluded from these risk estimates – one 31 CAG allele (565 sperm) and one 39 CAG allele (558 sperm)

| CAG Size | Frequency of Repeat Contraction Based on the CAG Size Range | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | Control | | Intermediate | | Reduced Penterance | | Full Penetrance | |
| | n | % | n | % | n | % | n | % | n | % |
| **27** | 103 | 3.5 | 103 | 3.5 | - | - | - | - | - | - |
| **28** | 69 | 4.1 | 34 | 2.0 | 35 | 2.1 | - | - | - | - |
| **29** | 60 | 3.6 | 8 | 0.5 | 52 | 3.1 | - | - | - | - |
| **30** | 105 | 4.5 | 17 | 0.7 | 88 | 3.8 | - | - | - | - |
| **31** | 95 | 7.3 | 7 | 0.5 | 88 | 6.8 | - | - | - | - |
| **32** | 40 | 7.1 | 1 | 0.2 | 39 | 6.9 | - | - | - | - |
| **33** | 147 | 9.2 | 0 | 0.0 | 147 | 9.2 | - | - | - | - |
| **34** | 563 | 14.6 | 9 | 0.2 | 554 | 14.4 | - | - | - | - |
| **35** | 275 | 12.0 | 5 | 0.2 | 270 | 11.8 | - | - | - | - |
| **Intermediate:** | **1457** | **8.0** | **184** | **1.0** | **1273** | **7.0** | **-** | **-** | **-** | **-** |
| **39** | 96 | 12.2 | 2 | 0.3 | 12 | 1.5 | 82 | 10.4 | - | - |
| **41** | 102 | 17.1 | 0 | 0.0 | 0 | 0.0 | 49 | 8.2 | 53 | 8.9 |
| **42** | 72 | 16.9 | 0 | 0.0 | 4 | 0.9 | 14 | 3.3 | 54 | 12.7 |
| **HD:** | **270** | **14.9** | **2** | **0.1** | **16** | **0.9** | **145** | **8.0** | **107** | **5.9** |

**Table 4.4 CAG-Size Specific Risk Estimates for Contraction Instability Based on the CAG Size Range**
The percentage of contracted sperm in the control (≤26 CAG), intermediate (27-35 CAG), reduced penetrance (36-39 CAG) and full penetrance (≥40 CAG) CAG size range is report as a percentage of the total number of sperm examined per CAG size as reported in Table 4.2. Two outliers were excluded from these risk estimates – one 31 CAG allele (565 sperm) and one 39 CAG allele (558 sperm)

| CAG Size | Frequency of Repeat Expansion Based on the CAG Size Range | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Total | | Control | | Intermediate | | HD | | Reduced Pentrance | | Full Penetrance | |
| | n | % | n | % | n | % | n | % | n | % | n | % |
| **15** | 5 | 0.4 | 5 | 0.4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **16** | 3 | 0.6 | 3 | 0.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **17** | 43 | 0.4 | 42 | 0.4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **18** | 10 | 0.6 | 10 | 0.6 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **19** | 7 | 0.7 | 7 | 0.7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **20** | 19 | 0.7 | 19 | 0.7 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **22** | 18 | 1.0 | 18 | 1.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **24** | 12 | 1.4 | 12 | 1.4 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **25** | 10 | 1.3 | 6 | 0.8 | 4 | 0.5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **Control:** | **127** | **0.6** | **122** | **0.5** | **4** | **0.1** | **0** | **0.0** | **0** | **0.0** | **0** | **0.0** |
| **27** | 58 | 2.0 | - | - | 54 | 1.9 | 4 | 0.1 | 4 | 0.1 | 0 | 0.0 |
| **28** | 27 | 1.6 | - | - | 25 | 1.5 | 2 | 0.1 | 2 | 0.1 | 0 | 0.0 |
| **29** | 37 | 2.2 | - | - | 36 | 2.1 | 1 | 0.1 | 1 | 0.1 | 0 | 0.0 |
| **30** | 65 | 2.8 | - | - | 59 | 2.5 | 6 | 0.3 | 4 | 0.2 | 2 | 0.1 |
| **31** | 73 | 5.6 | - | - | 66 | 5.1 | 7 | 0.5 | 4 | 0.3 | 3 | 0.2 |
| **32** | 32 | 5.7 | - | - | 29 | 5.2 | 3 | 0.5 | 0 | 0.0 | 3 | 0.5 |
| **33** | 160 | 10.1 | - | - | 146 | 9.2 | 14 | 0.9 | 6 | 0.4 | 9 | 0.5 |
| **34** | 479 | 12.4 | - | - | 387 | 10.1 | 92 | 2.4 | 81 | 2.1 | 11 | 0.3 |
| **35** | 481 | 21.0 | - | - | - | - | 481 | 21.0 | 464 | 20.3 | 17 | 0.7 |
| **Intermediate:** | **1,412** | **7.8** | **-** | **-** | **802** | **4.4** | **610** | **3.4** | **566** | **3.1** | **45** | **0.3** |

**Table 4.5 CAG-Size Specific Risk Estimates for Expansion Instability Based on the CAG Size Range**
The percentage of expanded sperm in the control (≤26 CAG), intermediate (27-35 CAG), reduced penetrance (36-39 CAG) and full penetrance (≥40 CAG) CAG size range is report as a percentage of the total number of sperm examined per CAG size as reported in Table 4.2. Two outliers were excluded from these risk estimates – one 31 CAG allele (565 sperm) and one 39 CAG allele (558 sperm)

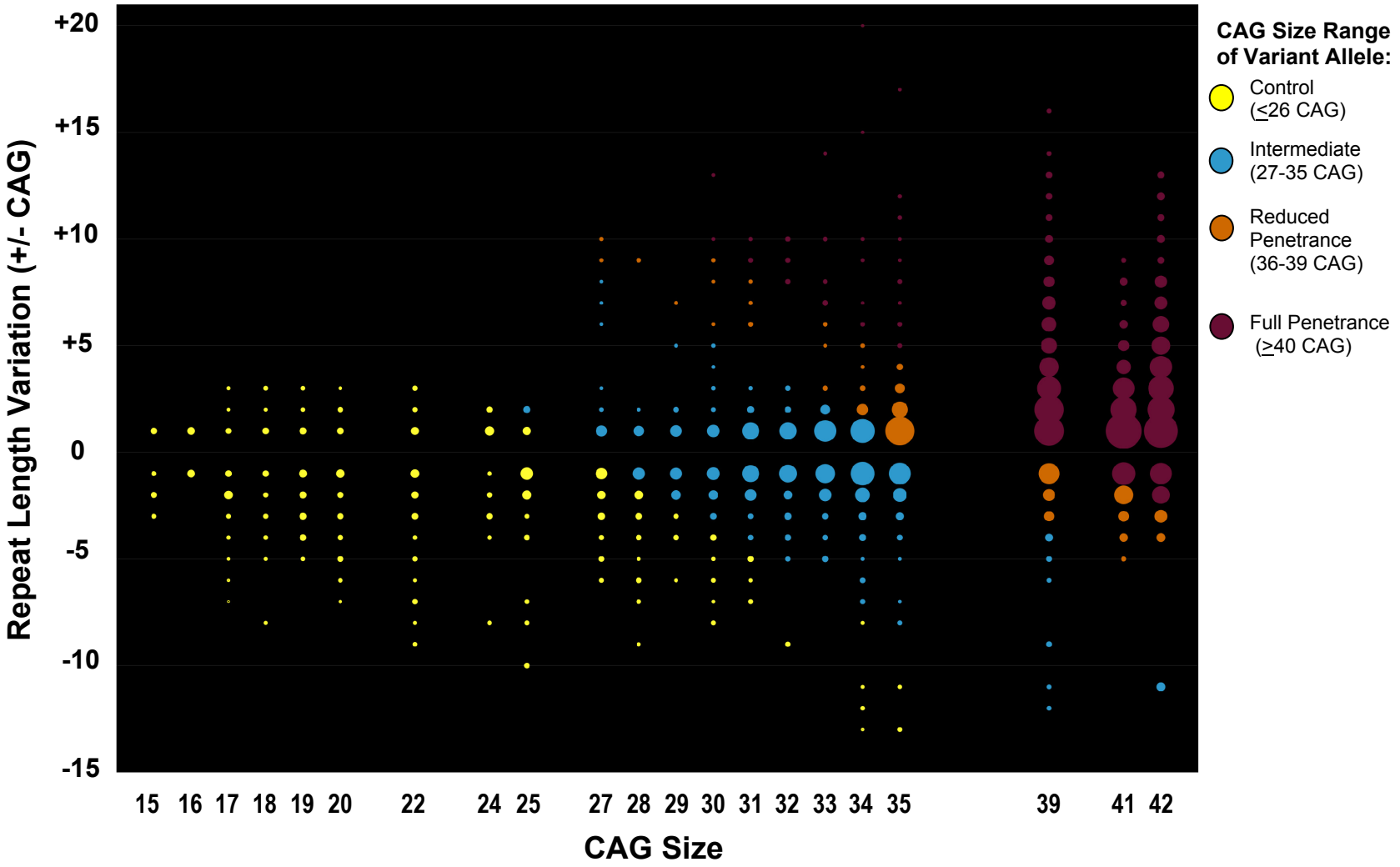



**Figure 4.4 Magnitude of Repeat Instability Based on CAG Size**

The magnitude of CAG repeat instability was quantified by the repeat length variation (RLV) between the progenitor and variant allele CAG sizes. The size of each dot is relative to the frequency of instability at a given RLV, with larger dots illustrating a greater frequency. The color of each dot corresponds to the CAG size range of the variant allele.

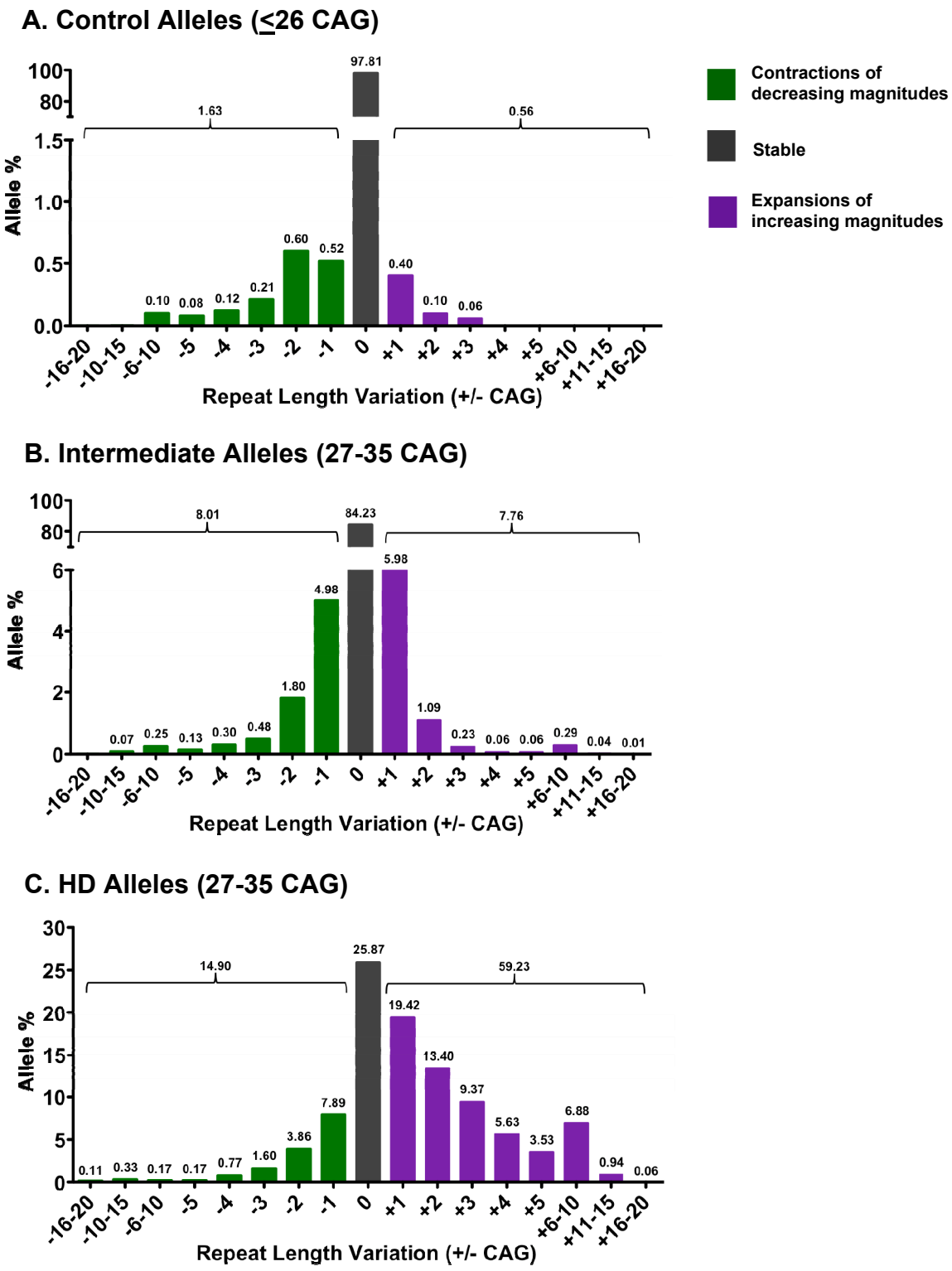



**Figure 4.5 Frequency of CAG Repeat Length Variation of Control, Intermediate, and Huntington Disease Alleles**
The percentage of alleles at each repeat length variation is report as a percentage of the total number of sperm examined per CAG size as reported in Table 4.2.

| | Haplotype | |
|---|---|---|
| | **Low-Risk** | **High-Risk** |
| **Control Alleles (≤26 CAG)** | | |
| Number of Alleles | 24 | 7 |
| Number of Sperm Examined | 15109 | 4819 |
| Mean CAG Size* | 17.4 | 21.0 |
| | *p=0.0002 | |
| **Intermediate Alleles (27-35 CAG)** | | |
| Number of Alleles | 8 | 18 |
| Number of Sperm Examined | 4580 | 11322 |
| Mean CAG Size* | 30.3 | 31.5 |
| | *p=0.3183 | |
| **HD Alleles (≥36 CAG)** | | |
| Number of Alleles | 2 | 1 |
| Number of Sperm Examined | 1216 | 597 |
| Mean CAG Size | 40.5 | 41.0 |

**Table 4.6 Summary of Control, Intermediate, and Huntington Disease Alleles Based on Haplotype**

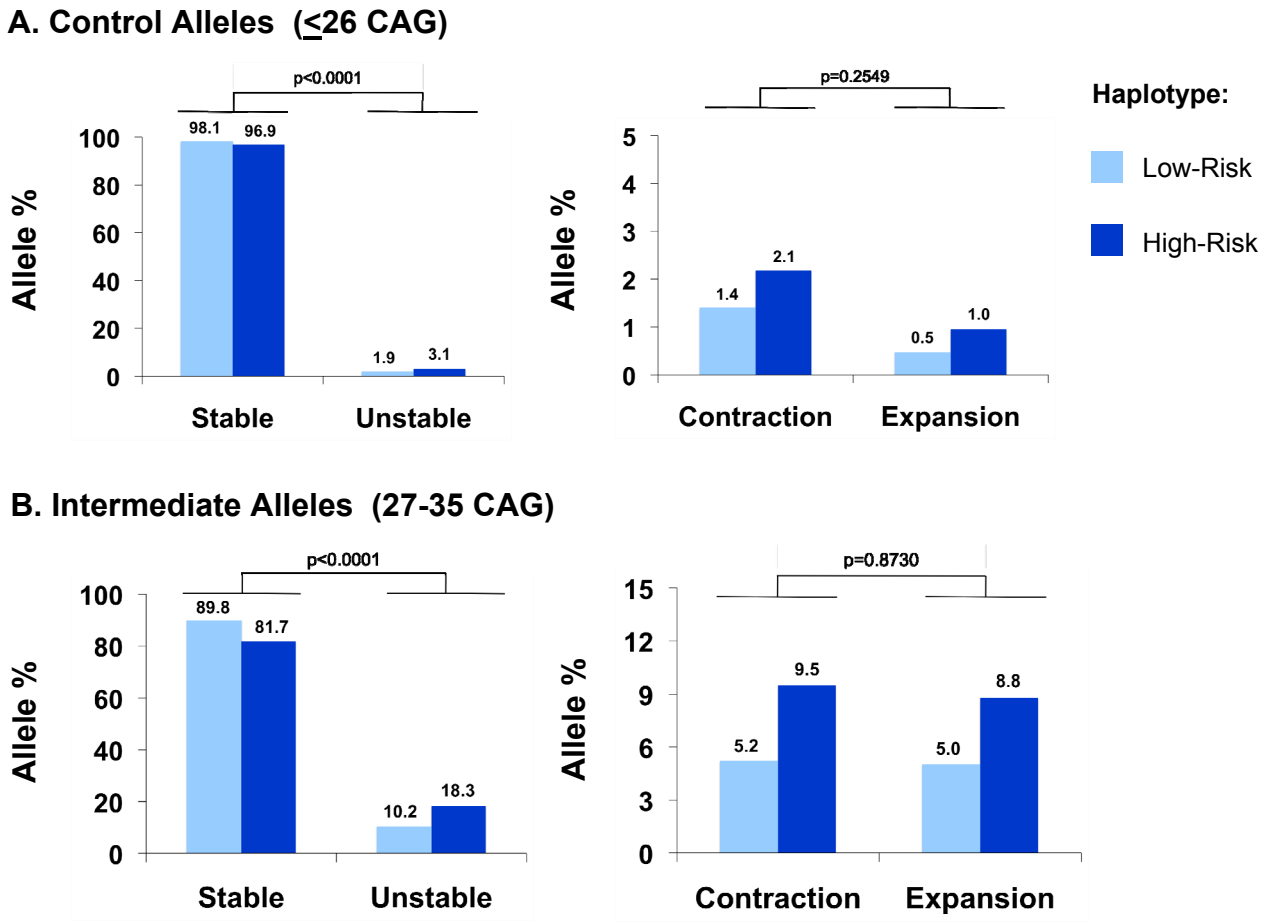



**Figure 4.6 Frequency of CAG Repeat Instability Based on Haplotype**
The percentage of control and intermediate alleles that were stable, unstable, contracted, and expanded is reported as the percentage of the total number of sperm examined per haplotype, which is reported in Table 4.6.

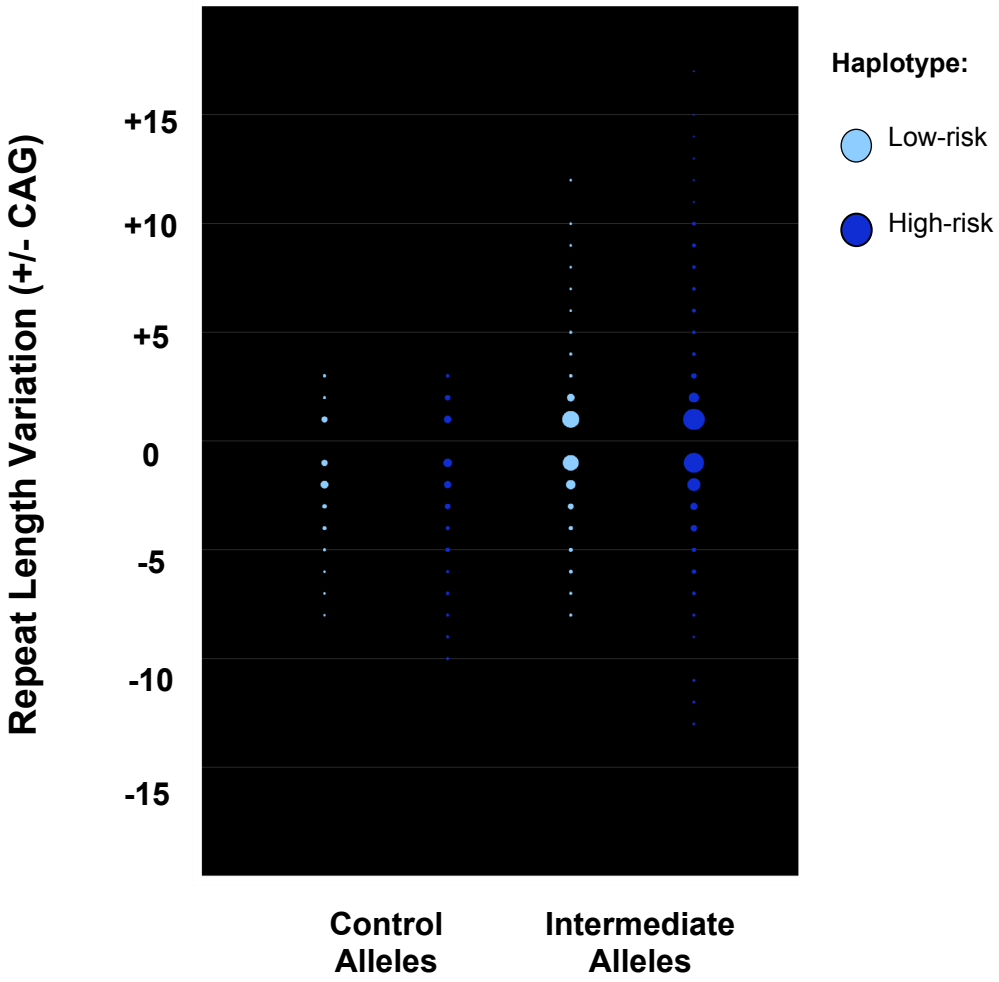



**Figure 4.7 Magnitude of CAG Repeat Instability of Control and Intermediate Alleles Based on Haplotype**
The magnitude of CAG repeat instability was quantified by the repeat length variation (RLV) between the progenitor and variant allele CAG sizes. The size of each dot is relative to the frequency of instability at a given RVL, with larger dots illustrating a greater frequency. The color of each dot corresponds to the haplotype of the allele

## 4.4 Discussion

This is the first formal study to examine the frequency and magnitude of germline CAG repeat instability of IAs and establish CAG size-specific risk estimates for repeat expansion into the HD range. The risk estimates generated are based upon 18763 sperm cells from 31 different IAs, representing the largest number of IAs and sperm to ever be examined. Our findings indicate there is a significant risk of new mutations for HD. While all CAG repeat sizes in the intermediate size range (27-35 CAG) were shown to expand into the disease-associated range ($\geq$36 CAG), the frequency of new mutations dramatically increased with increasing CAG size, underscoring the importance of CAG-size specific risk estimates. Alleles at the upper limit of the intermediate CAG size range had the highest risk of new mutations, with approximately 20% (n=481/2290) of 35 CAG alleles expanding into the HD range. The majority of new mutations were within the reduced penetrance CAG size range. In fact, full penetrance mutations were not observed until 30 CAG. The establishment of CAG-size specific instability rates will help inform more accurate risk assessment and genetic counselling.

Germline CAG repeat instability was observed at every CAG size examined, including control (n=35), intermediate (n=31), and HD (n=4) alleles. A significant ($p<0.001$) non-linear relationship was observed between CAG size and the frequency of repeat instability. While the frequency of instability was relatively low for control alleles, instability increased with increasing CAG size. In fact, the frequency of instability increased nearly 5-fold over the control CAG size range. Control alleles demonstrated a strong tendency to contract in CAG size, with the frequency and magnitude of repeat contractions exceeding expansions. Conversely, while only four HD alleles were examined, 75% of sperm were unstable and instability appeared to be highly biased towards repeat expansion. Within the intermediate CAG size range, the frequency of instability increased with increasing CAG, with repeat expansions showing the most prominent increase. The frequency of IA contractions outweighed expansions until the upper limits of the intermediate CAG size range. Collectively, these findings suggest there is a threshold length of approximately 33 CAG repeats

whereby there is a sudden increase in the frequency of repeat instability and a switch towards an expansion bias occurs.

The magnitude of repeat instability also showed a CAG length-dependent increase, where the frequency of small (1 to 3 CAG repeats) and large ($\geq$5 CAG repeats) repeat length variations increased with increasing CAG size. Control alleles predominately underwent small repeat length changes with a bias towards contractions. IAs displayed a relatively equal frequency of small repeat expansions and contractions but also underwent large expansions, albeit at a considerably lower rate. HD alleles demonstrated the highest frequency of large repeat expansions, although small repeat variations were still the most frequent. The magnitude of repeat instability observed across control, intermediate, and HD alleles is consistent with a step-wise model of expansion, whereby alleles undergo successive small expansion events over time into the HD range. This data also supports the observation of HD alleles undergoing extremely large repeat expansion that lead to juvenile HD.

This study provides important information on factors that influence CAG repeat instability. Multiple regression analysis indicated that together, CAG size, age and haplotype account for approximately 90% of the variance in the frequency of instability. CAG size was found to be the most significant predictor of CAG repeat instability, explaining 87% of the variance in the frequency of instability. The powerful influence of CAG size on repeat instability is highlighted when considering CAG size explains up to 70% of the variance observed in age of onset [Brinkman *et al.*, 1997; Langbehn *et al.*, 2004]. This data suggest that the size of the CAG repeat tract itself largely drives the frequency of instability. While there was a significant correlation between CAG size and haplotype ($p<0.001$), haplotype was not found to be a significant predictor of instability, although IAs found on high-risk haplotypes demonstrated increased frequency of instability compared to similar sized alleles on low-risk haplotypes. It is likely that the impact of haplotype is already accounted for by CAG size, given the underlying association between haplotype and CAG size.

While alleles on high-risk haplotypes had a higher rate of instability, haplotype did not impact the overall proportion of contractions or expansions. Control alleles on high-risk haplotypes were found to have a higher mean CAG size, which replicates previous findings [Warby *et al.*, 2009]. Control alleles on high-risk haplotypes also displayed a greater frequency of instability but this could be a reflection of their larger CAG size. Control alleles on high-risk haplotypes likely serve as a reservoir for expanded alleles because of their already large-normal size. This provides further support for a multi-step mechanism of expansion, whereby alleles containing the predisposing cis-elements undergo successive expansion events over time into the HD range [Warby *et al.*, 2009]. Consequently, all cases of HD may ultimately originate from a healthy individual who carried an allele predisposed to CAG repeat instability.

The results of the current study indicate a paternal age effect on the frequency of CAG repeat instability in HD. Paternal age has been shown to influence the likelihood of mutations in sperm for a number of genetic disorders including myotonic dystrophy [Monckton *et al.*, 1995] and achondroplasia [Wilkin *et al.*, 1998]. However, the impact of age on CAG repeat instability in HD is unclear. One study found males with an IA who were of advanced paternal age (average 37.5 years) demonstrated greater repeat instability, whereas age was not found to impact paternal instability in another study that examined HD alleles [Goldberg *et al.*, 1993b; Leeflang *et al.*, 1999; Wheeler *et al.*, 2007]. Future studies are necessary to explore more thoroughly the role of age in the process of CAG repeat instability. Studies may also aim to determine whether there is a paternal age-dependent threshold beyond which an increase in instability occurs.

While factors that influence CAG repeat instability have been identified in this study, approximately 10% of the variance in the frequency of instability remains unexplained. This provides strong support for unidentified genetic or environmental modifiers playing a role in repeat instability. Although haplotype can identify which alleles may be susceptible to repeat expansion, when the CAG tract will expand

appears to be more random [Warby *et al.*, 2009]. In the presence of a high-risk haplotype for repeat instability, *trans* genetic factors, such as DNA repair genes [Manley *et al.*, 1999] or unknown environmental features may impact when CAG expansion occurs. Differences in the frequency of repeat instability amongst siblings, with similar CAG sizes, has been speculated to be due to unknown genetic modifiers [Wheeler *et al.*, 2007] but whether these genetic modifiers act in *cis* or *trans* requires further study. Ancestral haplotypes for HD have been identified and therefore sequences closely linked to the *HTT* gene are shared amongst HD patients, which suggests that genetic modifiers in HD may be more likely to act in *trans* [Leeflang *et al.*, 1995; Wheeler *et al.*, 2007]. In fact, a recent study, which constructed detailed haplotype using SNPs located throughout the *HTT* gene and surrounding sequence, did not find an association between haplotype and age of disease onset [Lee *et al.*, 2012a]. This finding argues against the modification of these disease features by common *cis*-regulatory elements and supports the likelihood of *trans* genetic modifiers in HD.

The precise molecular mechanism underlying germline CAG repeat instability in HD remains elusive. While a variety of mechanisms have been proposed, slipped mispairing or the formation of secondary DNA structures during DNA replication is thought to be a critical step in the process of repeat instability [Cleary and Pearson, 2005; McMurray, 2010; Pearson *et al.*, 2005]. As the CAG repeat tract itself appears to have the largest influence on instability, perhaps the threshold length of instability observed in the sperm data is due to an increased tendency for single strand DNA to form stable secondary structures. The strong paternal bias for repeat instability in HD suggests that spermatogenesis may also play a role in the molecular mechanism. In fact, the paternal age effect observed in this study may be associated with an increase in the number of cell divisions during spermatogenesis as males age [Drost and Lee, 1995] but many questions remain about when instability occurs during spermatogenesis (i.e. the mitotic or meiotic cell divisions).

The occurrence of small (1 to 3 CAG repeats) and large (≥5 CAG repeats) repeat length changes in the present study suggests two distinct molecular mechanisms may underlie paternal instability in HD [Cleary and Pearson, 2005; Lenzmeier and Freudenreich, 2003; McMurray, 2010; Pearson *et al.*, 2005; Wells, 1996]. Small magnitudes of instability are commonly thought to occur as a result of DNA slipped mispairing during premeiotic replication in spermatogenesis [Goellner *et al.*, 1997; Leeflang *et al.*, 1999; Yoon *et al.*, 2003]. Conversely, large contractions and expansions may result from deficient Okazaki fragment processing and the formation of secondary structures during lagging- or leading-strand synthesis, respectively [Goellner *et al.*, 1997; Leeflang *et al.*, 1999; Yoon *et al.*, 2003]. Data indicates large repeat changes occur during the meiotic stage of spermatogenesis and may involve DNA repair mechanisms, such as base or nucleotide excision repair [McMurray, 2010; Monckton *et al.*, 1999]. Future research on the molecular basis of instability is required to better understand the nature of instability in HD. This knowledge may also identify unique avenues for therapeutic interventions.

This study is not without limitations. While the CAG-size specific risk estimates generated in this study will inform more accurate genetic counselling, these estimates are specific to the paternal germline. Quantified risk estimates for maternal repeat expansion are limited to familial transmission studies, which indicate the frequency of maternal instability is considerably lower than that observed in the paternal germline. The second limitation of this study is the assumption that sperm with expanded CAG repeat tract have an equal propensity to fertilize an ovum compared to sperm with smaller CAG lengths. It is possible that the length of the CAG tract may alter the sperm's viability and/or its ability to fertilize an egg. If sperm with an expanded CAG repeat tract have an altered fitness, these risk estimates may be an overestimate or underestimate depending on whether the expanded tract confers a decrease or increase in fitness, respectively. However, there is no data to suggest sperm carrying an expanded repeat tract have impaired fitness. Another assumption of this study is that all variants detected originated from the most common progenitor allele. We must acknowledge, however, the possibility that some

of the variants detected may have been derived from another variant allele. Consequently, the magnitude of instability may be incorrectly estimated in these cases.

The study findings have significant implications for genetic counselling. Given that every CAG size in the intermediate size range was shown to expand into the HD range upon transmission to the next generation, all individuals who have a CAG size between 27-35 CAG should receive comprehensive information and counselling on the clinical implications of an IA for offspring and future generations. Risk assessment for repeat expansion should be based on sex of the transmitting parent and CAG size. Males found to have an IA-PTR should be provided CAG-size specific risk estimates for repeat instability, particularly expansion into the disease range. The risk of paternal CAG repeat instability should also be discussed within the context of the magnitude of repeat expansion (i.e. into the reduced vs. full penetrance range). The relative nature of these instability estimates due unknown genetic or environmental factors modifiers could also be acknowledged during counselling. These quantified risk estimates will help inform accurate risk assessment upon which individuals may base their reproductive decision making.

# Chapter 5: "*Grasping the Grey*": Patient Understanding and Interpretation of an Intermediate Allele Predictive Test Result for Huntington Disease

## 5.1 Synopsis

Despite the characterization of IA almost 20 years ago, the predictive testing experience and psychosocial impact of receiving an IA-PTR has never been formally studied. Current genetic counselling practices regarding IA-PTRs and patient understanding of the clinical implications of an IA are also areas in which data is scarce. A single study has provided anecdotal insight into the clinical, psychological, and social experience of individuals who receive an IA-PTR and suggests these individuals experience confusion, uncertainty and guilt about the clinical significance of an IA-PTR [Maat-Kievit *et al.*, 2001b]. While genetic counselling practices regarding IA-PTR have not been formally examined, counselling in this regard has been described as challenging, particularly in relation to communicating the uncertain clinical implications [Tassicker *et al.*, 2006]. The difficulty experienced by medical genetics service providers is further compounded because the international predictive testing guidelines for HD do not yet acknowledge IAs [IHA and WFN, 1994].

Research is needed to explore individuals' understanding and perception of the clinical implications of an IA-PTR and identify the unique needs and psychosocial issues faced by these individuals and their families. Genetic counselling practices regarding IA-PTRs also need to be documented; specifically, what information on IAs is communicated to individuals and how and when this information is exchanged. Through in-depth interviews with individuals who have received an IA-PTR and medical genetics service providers, this study explored how individuals come to understand and interpret their IA-PTR by developing a theoretical model that explains this process. The overall aim of the study was to inform genetic counselling practices so that individuals who receive an IA-PTR receive accurate information and appropriate support and counselling for their unique psychosocial issues and needs.

## 5.2 Materials and Methods

This qualitative study aimed to explore individuals' understanding and interpretation of an IA-PTR using Strauss and Corbin's version of grounded theory, a methodology ideal for exploring social processes and interactions [Corbin and Strauss, 1990; Creswell, 2003; Strauss and Corbin, 1998]. Grounded theory emphasizes the systematic development of theory from data, thus the theory remains 'grounded' in the data, rather than being generated in the abstract. This qualitative methodology is characterized by simultaneous data collection and analysis; thus, is an iterative process of moving between data collection, analysis, and sampling based on the emerging theory. The process of understanding and interpreting an IA-PTR was examined from the perspective of the individual receiving an IA result, as well as the medical genetic professional providing genetic counselling for predictive testing for HD.

Grounded theory is an appropriate methodology for this research as it is commonly used to examine various processes in health care, such as medical decision making [Balneaves *et al.*, 2007; Howard *et al.*, 2011]. Being open-ended and flexible, grounded theory is an ideal methodology when little or no previous research has been performed on the topic to be studied [Morse *et al.*, 1996]. This methodology is also suitable because it emphasizes processes that occur and change over time, such as the process of predictive testing. Lastly, grounded theory has been recognized as an appropriate methodology for research in the field of genetic counselling because of its ability to generate evidence-based theoretical frameworks that can be used to inform clinical practice [Beeson, 1997; McAllister, 2001].

### 5.2.1 Theoretical Perspective

The theoretical perspective informing this qualitative grounded theory study is symbolic interactionism. A theoretical perspective is a philosophical viewpoint, which informs the chosen methodology of a study [Crotty, 1988]. In other words, the theoretical perspective describes the context, logic, and assumptions of the study, which will influence the course, focus, and ultimately, the outcome of the research.

Symbolic interactionism emphasizes that individuals come to make meaning about their world through an interpretative process of social interaction and considers social context fundamental to understanding human thought and action [Charon, 1985]. First described by the sociologists Mead [Mead, 1934] and Blumer [Blumer, 1969], symbolic interactionism views social interactions as dynamic, such that an individual's perceptions, understandings, and actions change over time as they encounter new experiences and information.

Based on the theoretical perspective of symbolic interactionism, the following three assumptions were made when interpreting and analyzing the data [Blumer, 1969]:

1) *"… human beings act towards things on the basis of the meaning that these things have for them…"* Thus, individuals act toward other individuals, objects, or situations based on the meaning that they have assigned to these things, instead of an inherent meaning.

2) *"… the meaning of such things is derived from and arises out of the social interaction that one has with one's fellows…"* Therefore, the meaning individuals assign other individuals, objects, or situations are socially constructed as individuals interact with others and their environment.

3) *"… these meanings are handled in, and modified through an interpretive process used by the person in dealing with things he encounters…"* Consequently, individuals assign meaning for other individuals, objects, or situations by first interpreting all the various meanings these things could have, as indicated by their interaction with others.

Symbolic interactionism is an appropriate theoretical perspective, as it highlights social interaction in the process of understanding and interpreting an IA-PTR. Predictive testing for HD does not occur in isolation; it involves an interaction between the individual and medical genetic service providers, including genetic counsellors and medical geneticists. This interaction will influence how an individual

comes to understand and interpret their PTR. Moreover, how an individual makes meaning about their PTR is also influenced by their interactions with their family, such as affected/unaffected family members, parents, significant other, and children and persons within the HD community.

### 5.2.2 Recruitment and Participants

A sample of 29 individuals who received an IA-PTR and eight medical genetics service providers, including genetic counsellors and medical geneticists, were recruited from four Canadian (Vancouver, Edmonton, Winnipeg, Toronto) and one Australian (Sydney) medical genetics clinics. Written documentation that the individual received genetic counselling about the clinical implications of their IA-PTR was required for study eligibility. Service providers eligible to participate routinely provided predictive testing for HD as part of their clinical practice. No other exclusion criteria were used for study eligibility. This open sampling was used to achieve a group of participants who had a diverse background in respect to their gender, age, education, family history, and time since receiving their PTR. Following open sampling, some participants (n=8) and service providers (n=2) were asked to take part in a follow-up telephone interview. These individuals were selected using theoretical sampling, which evolved the theoretical concepts by validating their properties, dimensions, and linkages and explored negative or discrepant cases in greater detail to ensure the developed theory accounts for variation. Participant recruitment and data collection continued until data saturation was reached whereby no new category emerged with further interviews, the same properties and dimensions of established categories were continually identified, and when the relationships between the various categories were established and validated.

Potential study participants were recruited through their medical genetic clinics by a mailed letter of invitation, a detailed study information sheet, and a consent form (Appendix 2). Written informed consent was obtained from all participants and medical genetics service providers. Ethical approval was received from all applicable university and hospital ethical review boards.

### 5.2.3 Data Collection Procedures

Semi-structured, open-ended interviews were conducted with study participants either in their home (n=14), place of work (n=7), or over the telephone (n=16). Interviews ranged from 45 to 90 minutes in length. Consistent with previous research, there did not appear to be a difference in the length or quality of interviews conducted face-to-face or over the telephone [Burnard, 1994; Sturges and Hanrahan, 2004]. Interviews were digitally recorded and transcribed verbatim. All interview transcripts were checked against recorded interviews for accuracy. Field notes were written immediately following the interviews to document important contextual and behavioral (i.e. participants' tone, inflection, and emotion) information that may be important to data analysis.

Four interview guides (Appendix 2), highlighting key issues to be explored with participants, were used during this study. The interview guide was continuously refined throughout the study based on the analysis of previous interviews, in order to ensure examination of relevant theoretical concepts and linkages. Initial open-ended interview questions were quite broad and designed to explore participants' experience with HD and predictive testing, their understanding of HD and their PTR, and their perception of the significance of HD and the impact of the result on their life and their family members' lives. Despite evidence that participants' received post-test counselling on the clinical implications of their IA-PTR, pilot research suggested that some individuals did not understand that they received an IA-PTR or were uncertain about its clinical implications [Semaka *et al.*, 2006]. Therefore, in an effort to avoid educating participants on their IA-PTR prior to exploring their understanding, the study information sheet, consent forms, and interview questions did not specifically refer to IAs or describe the clinical implications of an IA PTR in any way. As the data analysis progressed, interview questions were refined and became more specific to capture emerging and important concepts and develop conceptual linkages. Follow-up interview guides included questions to ensure data saturation was achieved in all study concepts and to confirm that participants'

experiences were accurately reflected in the developed theory. At the conclusion of each interview, participants' well being was assessed and further education, counselling, and/or support was offered on behalf of a genetic counsellor at their respective medical genetics clinic.

Two interview guides (Appendix 2) were used for interviews with medical genetics service providers. Questions in the first interview guide focused on their experience providing predictive testing for HD, their pre and post-test clinical practices regarding IAs, and the challenges they experienced in this regard. The second interview guide contained additional questions regarding the important theoretical concepts identified in the participant interviews.

### 5.2.4 Data Analysis Procedures

The qualitative software NVivo 4.0 [QRS International] was used to store, organize, and manage the interview data. The constant comparative method, a fundamental procedure in grounded theory data analysis, was used throughout the analysis. During the constant comparative method, data from each participant was continuously compared and contrasted against each other. Comparing the data in this manner allowed the theory to account for as much variation as possible, thereby increasing the applicability of the theoretical model, which is essential since the model will be used to inform genetic counselling implications. Throughout the analysis, written memos were used to capture thoughts, ideas, and decisions regarding the data and the emerging theory

The three sequential coding procedures of Strauss & Corbin's version of grounded theory, open coding, axial coding, and selective coding, were used in the analysis of the interview data. As the analysis progressed through the various coding procedures, the level of abstraction of the categories increased. The analysis began with line-by-line open coding where discrete incidents, ideas, events, or acts were given a descriptive label or code. Some example descriptive codes used in this stage of the analysis were as follows: *having a family history of HD*, *being unfamiliar*

*with HD*, *knowing HD inheritance is 50:50*, *receiving an unexpected result, feeling uncertain about children's risk*. This process fractured the data into important concepts discussed by participants.

During axial coding the descriptive labels were grouped and condensed, using the constant comparison method, into categories with specific properties and dimensions. An example category was *family experience* which ranged from extensive to limited depending on the individual's family history of the disease and their social and geographical circumstances. A coding framework of categories was developed and applied to all previous and subsequent interviews to identify recurrent categories discussed by participants. Relationships amongst categories were also explored, compared, and contrasted during the second stage of analysis using the coding paradigm, whereby the context, conditions, consequences, and actions & interactions in the data were systematically examined.

The final analytical coding procedure used was selective coding. During selective coding, the relationships between categories were modified and verified. Once these theoretical links were established, a core category was developed that encompassed the main categories in a cohesive theoretical model. The core concept in this study was the struggle individuals experienced understanding and interpreting their IA-PTR, which was conceptualized as *"Grasping the Grey"*.

### 5.2.5 Rigor

The principles used to evaluate the rigor of qualitative research differ substantially from the standards applied to quantitative research. Unlike the traditional notions of validity and reliability, the rigor of this qualitative study was assessed using the cannons of rigor developed specifically for studies using Strauss and Corbin's grounded theory methodology [Corbin and Strauss, 1990]. These cannons include generalizability, reproducibility, and precision. Generalizability refers to the degree to which the study results can be extrapolated to other circumstances; reproducibility is the degree to which the results can be replicated; and precision refers to the

explanatory power of the developed theory or the degree to which the theory will explain or predict what might happen in a given situation.

Methods employed to support study rigor included the use of the constant comparison method and widespread, systematic theoretical sampling such that a range of conditions and variations are built into the theory. Negative or discrepant cases were also actively accounted for throughout the analysis. The use of reflexivity and an audit trail of written memos detailing assumptions made, decisions taken, and meanings interpreted during the development of the theory also supported the rigor. Additionally, member checking with participants and medical genetics service providers was used, whereby the evolving theoretical concepts and their linkages, in addition to the final theoretical model, were presented to individuals throughout the analysis to determine if they felt it is an accurate representation of their experience.

## 5.3 Results

### 5.3.1 Participant Characteristics

A total of 29 participants who received an IA-PTR participated in this study. Both males (n=11, 38%) and females (n=18, 62%) were interviewed, with an overall mean age of 52 years (range 22-78 years). No participants displayed clinical symptoms of HD. The majority of participants were married (n=21, 72%) and had one or more children (n=22, 76%). On average, they received their IA-PTR 10 years ago (range 1-16 years). Of the 29 research participants, 17 were counselled by a medical geneticists or genetic counsellor who also participated in this study.

A total of eight medical genetics service providers were also interviewed. The majority of service providers were female (n=7, 88%). Over half of the service providers were genetic counsellors (n=5, 63%). On average, they had been providing predictive testing for HD for 10 years (range 2-20 years). All service providers had experience providing genetic counselling for IAs, however, the exact number of cases was not specifically ascertained.

Additional demographic characteristics of the study participants and service providers are provided in Table 5.1.

### 5.3.2 Overview of the *"Grasping the Grey"* Theoretical Model

The "*Grasping the Grey*" theoretical model refers to the process individuals experienced in understanding and interpreting their IA-PTR. Both participants and medical genetics service providers commonly referred to an IA-PTR as a *"grey"* result to describe both its uncertain clinical implications and its inherent uncertainty due to limited scientific knowledge. One participant explained:

> *"Where I sit with thirty-five [CAG repeat], while it's okay for me, the grey area comes in for future generations and what's going to happen to them. It's not clear what the future holds for my future generations, it's extremely grey."*

Another participant said:

> *"It's an offbeat number, it is sort of some crazy molecule that is not really well understood. It's grey, there's a lot of unknowns."*

The core concept in this theoretical model was the struggle participants experienced in understanding the clinical implications of their IA-PTR and interpreting its significance for their life and the lives of their family, specifically their children and grandchildren. The difficulty participants experienced in the process of understanding the meaning of their IA-PTR was conceptualized as *"Grasping the Grey"*. A genetic counselor explained:

> *"Regardless of whether it's an intermediate allele from the general population or from a new mutation [family], all patients seem to struggle with this result and what the risks are to their kids and grandkids. In my experience most [patients] have a tremendously difficult time understanding intermediate alleles and the impact it will have [on their lives]."*

A participant shared:

> *"I struggle with what is [this result] going to do to my children and even their potential children and also, there's a lot of unknowns about this [result] so I definitely think a lot of us struggle with that too."*

Several major categories were identified as playing an important role in the *"Grasping the Grey"* theoretical model, including the participants' family experience,

beliefs about the genetics of HD, expectations of predictive testing and the pre-test genetic counseling they received (Figure 5.1). These categories interacted to impact the degree to which participants struggled to understand and interpret their IA PTR. More specifically, individuals' beliefs about HD inheritance were largely a consequence of their familial experience with HD. Moreover, the pre-test genetic counseling individuals received was also informed by their family history. Together, participant's beliefs and pre-test genetic counseling created their predictive testing expectations. Collectively, these categories and their theoretical linkages influenced how participants' understood their *"grey"* PTR. The understanding participants developed about the clinical implications of their IA result became the foundation upon which they reflected and interpreted its significance and impact on their lives.

The individual categories of the "*Grasping the Grey"* theoretical model vary along a continuum. The extremes of these continuums are described to explain how individuals came to understand and interpret their *"grey"* PTR. While the number of participants in each category of this process is reported in Table 5.2, the *"Grasping the Grey"* model was dynamic and continuous, as such where participants fell along each continuum shifted over time in response to new information and experiences.

### 5.3.3 Family Experience

Participants' family experience with HD significantly influenced their understanding and interpretation of an IA-PTR. The two different familial contexts in which an IA can be identified, a new mutation family or a family with a long-standing history of the disease, created two different familial experiences –*"out of the blue" and "growing up with HD"* (Figure 5.1). Participants' family experience was shaped by their exposure to HD, including their age when they were first exposed to HD, the number of affected persons in their family and their relationship to the participants (i.e. affected sibling, parent, extended family members), and their social and geographical proximity to affected family members. Although several participants in both types of family experience spoke of providing care for family members affected

with HD, they did not overtly link this responsibility to their understanding and interpretation of an IA-PTR.

A total of 14 research participants experienced HD *"out of the blue"*, of which 10 had an elderly parent and four had a sibling unexpectedly diagnosed with HD. No participants who experienced HD unexpectedly had a family member previously identified with an IA. The remaining 15 participants had a *"growing up with HD"* family experience. For three of these participants, a family member, either a sibling or parent, was previously identified as having an IA in the context of a long-standing family history (Table 5.2).

#### 5.3.3.1 *Out of the Blue*

Participants who experienced HD *"out of the blue"* had a new mutation family history. Most often, these individuals inherited an IA that previously underwent CAG repeat expansion into the HD range upon transmission to their affected family member. Most often, the first time these participants were exposed to the disease was when they were adults and either their sibling or elderly parent was unexpectedly diagnosed with HD. These participants had no previous exposure to HD prior to the sudden diagnosis of their parent or sibling. Many of these participants had no previous knowledge of HD, one individual shared:

> *"We were just dazzled because we'd never heard of HD before. I mean I'd heard of [HD], but I'd never heard of it in my family."*

Participants often described a specific moment when they first began noticing their family members unusual behavior and speculating on potential causes of the symptoms. One woman said:

> *"When my mother came [to Canada], I noticed it right away, that there's something wrong with her mouth, with her facial muscles and expressions. I was thinking at that moment, because she has dentures, that the dentures were not properly done and they were bothering her so she was doing that movement."*

For these families, the process of obtaining an HD diagnosis was challenging. Some families struggled for many years, through multiple diagnoses, before HD was definitively diagnosed. The absent family history or late age of onset likely acted as barriers to the diagnostic process. One participant shared:

> *"When my father began to exhibit symptoms, it took forever, maybe five or six years, before he was [diagnosed] and I think because of his age, he was in his seventies, people were not thinking Huntington's."*

Another participant recalled:

> *"[My sister's] first diagnosis was actually that she had Tourette's [syndrome] and then she got progressively worse with the falling so we proceeded taking her to another doctor and yet another doctor until finally we went [back] to the family doctor and he arranged for the Huntington's blood work to be done."*

These participants expressed shock and disbelief at the diagnosis of HD in their family. They struggled to understand how HD could be an inherited disease when it occurred in their family without a previous history:

> *"I was shocked and I suppose in a way I didn't believe it at first, even though we had the positive diagnosis, I just started to question [the diagnosis], like we don't have a family history of HD, so it can't be? How could [my mother] have this when we haven't seen it in any other family members?"*

In an effort to reconcile the contradiction of an inherited disease occurring in their family with no previous history, some individuals discussed searching their family history for evidence of HD. One man said:

> "*We went back to the family tree, based on church records, to about like 1600 and there was never any Huntington's. Of course they wouldn't recognize it [as HD] then but there was nothing unusual."*

#### 5.3.3.2 *Growing Up with Huntington Disease*

Participants who had a *"growing up with HD"* family experience had a long-standing family history of the disease. While these individuals had a family history of HD, the IA was most often inherited from their unaffected parent on the non-affected side of their family. They were frequently first exposed to the disease in childhood or adolescence and often had multiple affected family members, including parents, siblings, aunts/uncles, and/or grandparents. For a significant portion of their lives,

these participants knew that there was *"something"* in their family; however, many only received the label of HD in the last 15 to 20 years. One male participant shared:

> "*I've seen my grandfather go through it when I was just in elementary school and I thought it was the most devastating thing, and now I've seen my aunt and two of my uncles die from it too."*

Another participant explained:

> *"I was about thirty when I became aware of quote 'Huntington disease', but I was a young teenager when I first started to see the impact of it [on] my family."*

Participants often shared vivid memories about their experiences with affected family members when they were younger:

> "*I can remember as a child driving with my uncle and sort of being afraid because he was driving and he was shaking and turning and sort of carrying on. I remember sitting in the car and not feeling safe driving with him."*

As a consequence of these profound family experiences, many participants lived in fear of both the disease's symptoms and its genetic implications:

> *"My mom was in a bed and even though the sides came up on it, they'd find her on the floor in the morning, her movements were that violent. I mean that's scary, really scary."*

Another participant shared:

> *"It's frightening, very frightening. It's like almost every year we're hearing that somebody else in the family has [HD]."*

Of the 15 participants who had a long-standing family history of HD, seven participants' exposure to HD was limited due to geographical or social circumstances. More specifically, some individuals had restricted contact with their family when they were adults because they were no longer living in the same city or country as their family. Other participants' family experience was minimized because of estranged family relationships. A number of participants' parents divorced when they were young and consequently they did not spend as much time with their affected parent and/or extended family members. For these participants, despite having an extensive family history of the disease, their familial experience had aspects, which resembled an *"out of the blue"* family experience. One participant shared:

*"When we found out we were pregnant, we figured that it would be a good opportunity to try to find my father because I had no contact with him for over twenty-eight years. We were able track him down and that's when he told us about the Huntington's family history and I was like, okay, Huntington's, never heard of it."*

### 5.3.4 Beliefs about the Genetics of Huntington Disease

Participants' beliefs about the genetics of HD played an important role in the *"Grasping the Grey"* process. Individuals' beliefs about HD were largely developed within the context of their family experience. As a consequence of either experiencing the disease "*out of the blue"* or *"growing up with HD"*, participants largely developed two different belief systems about the genetics of HD and how the disease is inherited – *"blank slate"* or "*black & white"* beliefs (Figure 5.1). Of the 29 research participants, 12 individuals had *"blank slate"* beliefs and 17 established "*blank and white"* beliefs (Table 5.2).

#### 5.3.4.1 *Blank Slate*

Individuals who experienced HD "*out of the blue"* were in the process of forming their beliefs about the genetics of HD. With limited family experience and knowledge, these participants did not hold any preconceived notions about HD, its inheritance pattern, and the resulting familial risks. Consequently, their belief system was like a '*blank slate'*. One woman explained:

*"I think that people who do have it in their family, they know [HD], they know how it works, but for us everything was brand new, we're like a blank slate, we just knew nothing about it."*

Another participant shared*:*

*"As soon as I knew [my sibling] had HD, I got an awful lot of information and just tried to find out as much as I could about it because I didn't know a thing."*

Individuals who had a "*blank slate"* belief system appeared to experience less difficulty understanding the clinical implications of their IA result. These participants were in the process of establishing their beliefs, given their "*out of the blue"* family experience, and expressed limited conflict with previous beliefs about HD when

discussing their IA-PTR. The meaning of IAs appeared to be more easily incorporated into their developing belief system about the disease.

#### 5.3.4.2 *Black & White*

Individuals who grew up with HD had a well-established belief system, which developed over time as a result of their profound familial experiences, which included conversations with their family members and *"watching"* HD being inherited in their family. Educational resources, such as pamphlets from community HD organizations and internet sites on HD, also help ingrain a particular set of beliefs about the genetics of HD. These individuals believed that the genetics of HD is *"black & white".* They believed that HD is an inherited disease that does not *"skip"* generations. In other words, these participants believed that an individual must have a family history in order to develop the disorder; children were only at-risk if one of their parents has HD. One woman explained:

> *"It's black or white, we each have that 50% chance of getting it, and it never skips a generation, sometimes a disease will skip [a generation] but HD never skips a generation."*

Another participant said:

> *"The way [HD] kind of works is if your father has it then, you have that 50% chance but if he doesn't have it, then you're in the clear."*

In contrast to those participants who held "*blank slate"* beliefs, many participants who held "*black & white"* beliefs appeared to experience great difficulty grasping the meaning of their IA predictive test result. During the interviews, these individuals struggled to understand the uncertain clinical implications of an IA and reflected on how this new knowledge conflicted with their firmly entrenched belief about how HD is inherited. As one knowledgeable participant explained:

> *"If you don't develop Huntington's, your kids won't develop Huntington's, normally that's true but not with this [result]."*

Another participant said:

> *"With this result, it's like we're the exception to the rule."*

### 5.3.5 Pre-test Genetic Counselling

All participants in this study received post-result genetic counselling about the clinical implications of an IA-PTR. However, the pre-test genetic counselling participants received differed and this played an important role in the "*Grasping the Grey"* theoretical model. Medical genetics service providers indicated that they addressed IAs in every pre-test counselling session when discussing the CAG repeat continuum. However, the amount of information, time, and emphasis placed on the possibility of an IA-PTR varied based on the individuals' family history. The genetic counselling participants received not only influenced their beliefs but also shaped their predictive testing expectations. Two types of pre-test genetic counselling were identified – *"ABC"* and "*50-50"* (Figure 5.1). Of the 29 research participants, eight individuals described what was categorized as in this study as *"ABC"* genetic counselling and 21 persons described *"50-50"* counselling (Table 5.2).

#### 5.3.5.1 *ABC*

Participants who received *"ABC"* pre-test genetic counselling largely presented with a new mutation family history when their sibling was diagnosed "*out of the blue*" with HD. However, four participants who had a *"growing up with HD"* family experience also received *"ABC"* genetic counselling given that an IA was previously identified in their family. While these individuals had a family history of HD, an IA was identified most often on the non-affected side of their family. During this type of pre-test genetic counselling, three possible PTRs were discussed with the same amount of emphasis and attention – mutation-positive (i.e. "*A"*), negative (i.e. "*B"),* and IA (i.e. "*C*") results. In many cases, information on IAs was also provided to explain how HD occurred in the individual's family with no previous history. A medical genetics service provider described this counselling practice:

> *"A family history where I might give intermediate alleles more face-time or discussion time is if somebody comes in and they have a sibling who's affected and their parents have no signs or symptoms."*

Reflecting on the genetic counselling they received, a participant shared:

> *"[My genetic counsellor] said there were basically three result options, A, B or C."*

Individuals who received *"ABC"* pre-test genetic counselling described being able to easily accept and understand the meaning of their IA result. The additional education on, and preparation for, this result possibility, combined with their *"blank slate"* beliefs, likely assisted them in understanding their *"grey"* result and the development of a belief system that incorporated IAs.

#### 5.3.5.2 *50-50*

When HD occurred in an individual's parent, genetic counsellors focused their pre-test counselling on the autosomal dominant, or "*50-50"* inheritance pattern of HD, and the possibility of either mutation-positive or negative PTRs. During this type of counselling, IAs were only briefly mentioned when discussing the CAG repeat continuum. Thus, the amount of information, time, and emphasis on an IA predictive test result was minimal. Instead, individuals were actively prepared for the 50% possibility of receiving their parent's genetic mutation. A medical genetics service provider explained:

> *"I'll have looked at the family history and if clearly the parent has a CAG repeat in the affected range I will use the language that it's '50-50'. I may mention a small possibility for an intermediate allele but that's a complicated thing so I try not to spend a lot of time on it."*

Reflecting on the genetic counselling they received, a participant shared:

> *"My [genetic] counsellor told me that I had a fifty percent change of having the [genetic] mutation and that it carried on to my children and they [would] have a fifty percent chance. I didn't know about this funny in-between result until later [when I got my result]"*

*"50-50"* counselling was the predominant pre-test genetic counselling and was provided to all participants who had an affected parent, including individuals who had a *"growing up with HD"'* family experience (n=12) and a number of individuals who experienced HD in their family *"out of the blue"*, when an elderly parent was unexpectedly diagnosed (n=10). For participants who grew up with HD and

developed *"black & white"* beliefs, this type of pre-test genetic counselling reinforced their beliefs, which conflicted with the clinical implications of an IA, and likely intensified their struggle to understand and grasp the meaning of their result. For the participants who first experienced HD unexpectedly in an elderly parent and held "*blank slate"* beliefs, this pre-test genetic counselling supported the formation of a *"black & white"* belief system. In contrast to individuals who held well-established *"black & white"* beliefs, these individuals appeared to struggle to a lesser degree in understanding their *"grey"* result.

### 5.3.6 Predictive Testing Expectations

Participants' expectations about predictive testing played an integral role in the "*Grasping the Grey"* theoretical model. Individuals' beliefs about the genetics of HD and the pre-test genetic counselling they received interacted to create expectations of what PTRs were possible and the degree to which these results would relieve their uncertainty about their genetic status and its consequences for their children. Participants had either *"option C"* or *"yes or no"* predictive testing expectations (Figure 5.1). Of the 29 research participants, six individuals had *"option C"* expectations and 23 had *"yes or no"* expectations (Table 5.2).

#### 5.3.6.1 *Option C*

Only a minority of individuals had the expectation that they could receive a *"grey"* PTR that would have uncertain implications for their children. Individuals largely formed *"option C"* predictive testing expectation because they received "*ABC"* pre-test genetic counselling. One participant whose sister was diagnosed "*out of the blue"* explained:

> "*I knew the three [result] possibilities were that I wouldn't have the mutation, [that] there was this intermediate area of numbers, and then of course, there was [a chance] I would get [HD]."*

Another participant shared:

> *"I knew before [receiving my result] that there was a third option."*

*"Option C"* predictive testing expectations likely minimized the difficulty participants experienced when grasping the meaning of their IA-PTR. With the expectation that a *"grey"* result was possible, participants did not experience intense shock at receiving this result and described conversations with their genetic counsellors in which they were able to hear, comprehend, and interpret the information being provided about their IA result.

#### 5.3.6.2 *Yes or No*

The majority of individuals in this study expected predictive testing to provide a "y*es or no"* answer about whether or not they had inherited the genetic mutation and would eventually develop HD. They did not anticipate the possibility of receiving a *"grey"* result. In fact, many individuals indicated they had never heard of an IA before, despite IAs being mentioned in their pre-test counselling session when discussing the CAG repeat continuum. One older man shared:

> *"They threw me with the third option, rather than the yes or no. I thought that you either had it or didn't."*

These participants were also not aware that PTR could have uncertain implications. Instead, they believed predictive testing would provide them clear, definitive information. One woman shared:

> *"When the news came out, it wasn't as clear cut as I thought it would be."*

Another participant explained:

> *"My expectation of [medical] tests in general, is that testing is an like an on-off switch, you don't generally experience a grey area; you have strep throat or you don't; you are pregnant or you're not; and if you're going for [HD] genetic testing, you're looking at a definitive answer."*

These individuals expressed intense shock at receiving a PTR that differed from their expectations. One woman explained:

> *"You're in shock [because] it's not the answer you're expecting. You're going in expecting 'a or b' and then someone gives you a 'c' choice, which is not at all what you were expecting."*

Participants were also surprised by the clinical implications of an IA and that HD could *"skip"* generations. One male participant with a young child explained:

> "*I was just stunned to find out that there was actually a possibility that our child could develop it. I never considered that a possibility unless I was [mutation-] positive so I was just blown away that there was actually a risk that my child could develop it."*

As a consequence of feeling shocked, many of these participants reported *"shutting down*" after receiving their IA-PTR. This reaction likely made it difficult for individuals to hear the information being provided about an IA during their post-result genetic counselling. One female participant explained:

> *"I think when you hear that [IA] result, they tell you what it means and you're listening but you're not really hearing so when you go home, you think, 'What did they say?' I was listening but not really absorbing what they were saying to me."*

The reaction of *"shutting down"*, combined with *"yes or no"* predictive testing expectations, possibly became barriers to participants' ability to process and understand the novel information being provided about their IA-PTR and likely contributed to their struggle to understand and interpret their *"grey"* result.

### 5.3.7 Understanding of an Intermediate Allele Predictive Test Result

Participants' understanding of their IA-PTR consisted of their knowledge of its clinical implications and surrounding scientific facts, such as the occurrence of new genetic mutations, general population IAs, CAG repeat instability, and the impact of gender on the risk of CAG repeat expansion. Individuals' understanding, particularly regarding the clinical implications of an IA, varied along a continuum of poor (n=6), uncertain (n=8), and good (n=15) understanding (Figure 5.1, Table 5.2).

Participants with poor understanding believed that since they would not develop HD, their children were no longer at-risk to develop the disease. In other words, these participants' understanding reflected the clinical implications of a mutation-negative result. Importantly, these individuals were certain in their understanding and did not perceive themselves as having misunderstood the clinical implications of their predictive test result.

Other participants in this study expressed uncertainty about their understanding. This uncertainty was expressed either about their own risk to develop HD or about the clinical significance of their result for their children. Uncertainty fell into two categories, actual uncertainty or perceived uncertainty. More specifically, individuals who expressed actual uncertainty were genuinely uncertain about the clinical implications of their IA result. While these participants sensed that their result was not *'a straight negative',* they could not articulate the significance of this. One participant said:

> *"I don't understand what [my result] means. Does it mean I have Huntington's because I'm a thirty-four? I really honestly don't know at this point; I'm a little confused."*

Another participant shared:

> "*I don't have Huntington's, I understand that much. [But] the kids, can they or can't they… will they or won't they? I'm not sure if it means they're safe or not?"*

Other individuals perceived themselves as being uncertain about the clinical significance of their *"grey"* result but in reality, these participants had good understanding. One participant stated;

> *"My result means there is a small chance my kids could still develop HD, but I could be mistaken, I'm not really sure, I don't really know."*

Another group of participants in this study had good understanding about the clinical implications of their result for themselves and their children. These individuals understood that while they would not develop HD, their children or future generations of their family remained at-risk of the disease. These individuals were also aware of the inherent uncertainty due to the limited scientific knowledge that currently exists about IAs.

Participants' understanding of the scientific facts surrounding IAs was also variable. Many individuals did not understand why a risk remained for their children. Specifically, participants had difficulty grasping the concept of repeat instability and how expansion of their IA could result in their children developing HD later in life. One participant, with good understanding, explained:

> *"I think the concept of [CAG repeat] instability is something that people really have to get a hold of, just because you're not going to get [HD], instability exists and therefore your children can still develop it. Your [repeat] number can jump and expand if you're in the grey area."*

Many participants also struggled to understand from whom they inherited the IA. In other words, some individuals had difficulty understanding that they received their IA from their unaffected parent or the non-HD side of the family. One individual explained:

> *''Maybe if people really thought about it then they would be conscious of the fact that [the intermediate allele] could come from the other side [of the family] but you kind of disregard the side [of the family] where [HD] isn't exhibited. I mean I didn't give any consideration to that [possibility] so I was really surprised."*

Understanding the scientific facts surrounding IAs played an important role in the "*Grasping the Grey*" theoretical model as it appeared to assist many participants in feeling more certain about their understanding of the unusual clinical implications of an IA result.

### 5.3.8 Interpretation of an Intermediate Allele Predictive Test Result

The interpretation of a *"grey"* PTR refers to participants' perception of the significance the result will have in their life and the lives of their children. Participants' interpretations were highly influenced by their understanding of the clinical implication and scientific facts of IAs. Individuals' interpretations occurred within the context of their family experience, beliefs, genetic counselling, and expectations and evolved over time, shifting in response to new experiences and knowledge. Four different interpretations of an IA-PTR were described by participants: six individuals interpreted their result as *"free & clear"*, eight individuals were *"sitting on the fence",* 10 individuals interpreted their result as something that *"could be worse"*, and five individuals view their result as meaning their family had a *"threatened future"* (Figure 5.1, Table 5.2).

#### 5.3.8.1 *Free & Clear*

Some individuals interpreted their IA-PTR to mean that they and their family were "*free & clear*" of HD. These individuals formed this meaning based on their poor understanding of the clinical implications of an IA result. One male participant who had a long-standing family history shared:

> *"I'm free and clear and my children are even better because it's 50-50."*

Another participant shared:

> *"As far as my kids go, because I don't have it, they can't have it, so it's no use them getting tested, for us, it's just bygones."*

While none of these individuals were aware of the clinical implications associated with their IA-PTR, a small proportion were aware of some of the scientific facts surrounding IAs. This included knowing that their CAG size was on the "*border"* or higher than normal or that they inherited their gene from the *"wrong"* parent or non-affected side of the family. The majority of these individuals dismissed the significance of these facts. For example, they downplayed the fact that their non-affected parent carried an *"HD gene"* because *"we all carry something*". One older male participant explained:

> "*The amazing thing was that [the geneticist] said my [unaffected] mother had the gene too. How they figured that out, I don't know, because she was long since dead, but that wasn't really a big thing, it was just like, it's on your father's side and oh by the way, your mother had a strain of it too."*

#### 5.3.8.2 *Sitting on the Fence*

Many participants in this study were *"sitting on the fence"* about the meaning of their IA-PTR because they had uncertain understanding about its clinical significance. In other words, the uncertainty these individuals experienced hampered their ability to fully interpret the meaning of their result for themselves and family. Consequently, these participants persisted in a state of uncertainty about the meaning of their IA-PTR because they perceived themselves as having an inadequate level of understanding upon which they could establish meaning. One individual shared:

> *"When I got an intermediate, [the geneticist and genetic counsellor] were like, oh, we don't really know much about this [result]. So that left me kind of sitting*

*on the fence thinking, you're telling me I'm not positive, you're telling me I'm not negative, instead you're telling me I fall in the middle but you don't really understand what that means. Well, guess what? Neither do I!"*

Another individual with two children explained:

"*[The genetic counsellor] referred to the results as black, grey, and white. I think for my sisters and myself, I mean we still say it now, 'Thank God we're in the grey area' yet we don't really know what being in the grey area means."*

#### 5.3.8.3 *Could Be Worse*

Another group of participants interpreted their IA-PTR as something that *"could be worse"*. These individuals understood that while they would not develop HD, a risk remained for their children or grandchildren. They perceived themselves to be "*lucky*" to have received a *"grey"* result because the worst-case scenario had been avoided - they would not develop HD and their children's risk was considerably lower than 50%. These individuals used a comparative process whereby they weighed their children's risk to develop HD as a consequence of their IA-PTR against the 50% risk their children would have had if they had received a mutation-positive PTR. One participant said:

*"I felt lousy in a way, knowing that [my children] could possibly get it but then the way I understood it, it was a lot less chance that they would [get it], whereas in Huntington's you're 50-50 that you'd get it or not get it."*

Participants also compared the risk their children had to develop HD to other risks their children may encounter in life that are "*just as risky*". One female participant explained:

*"[My children] could get multiple sclerosis or autism. I would rather have what we've got than that. There are dozens of diseases and situations out there that are worse."*

As a result of this comparative process, these individuals did not perceive the risk to their children to be significant. However, many indicated that in the *"back of their mind"* they were concerned about their family's uncertain future with HD. One woman described:

*"It's just something that is kind of like a grey cloud that looms, that maybe I could have still have passed [HD] on [to my children]."*

Interestingly, the majority of participants who interpreted their result in this manner were female (n=7), likely reflecting their understanding that for females, the magnitude of risk to children is believed to be extremely low. One woman shared:

"*I do a lot of thinking [about] how lucky I am that although I kind of have the gene, it's not enough that I will actually get the disease, nor can I pass it to my children because I'm a woman."*

#### 5.3.8.4 *Threatened Future*

Several participants in this study interpreted their IA-PTR to mean that their family had a *"threatened future".* These individuals understood that while they were no longer at-risk of developing HD, they believed HD was a significant threat for their children and future generations of their family. All participants who perceived their family as having a *"threatened future"* were males, likely reflecting their understanding of the role of sex on the risk of IA expansion. They lived with the knowledge of their children's uncertain future in the forefront of their mind and many thought about the clinical implications on a daily basis. One male participant shared:

*"As much as I'd like to say we don't think about [my result], I don't think a day goes by that [my wife and I] wouldn't think about it. We're always thinking, is [our son] going to be affected? Are [our] grandkids going to be affected? We're just praying everyday that Huntington's is out of our family."*

For many participants, this interpretation of their IA-PTR led to much fear, anxiety, and guilt:

*"Any possibility that I had inflicted this on my daughter was just enormous, it really wouldn't matter what the percentage was. The legacy you want to give your child is values, education, ability, everything in the world; the one legacy you don't want to give your child is a genetic disease that will kill them.*

A *"threatened future"* interpretation appeared to have the greatest impact on participants' reproductive decision making. Of the five males who interpreted their result in this manner, three had the desire to have children either in the near or

distant future. The other two participants were older, having received their IA-PTR after completing their family. All males who were considering having children expressed great concern over the potential risk of transmitting an expanded allele in the HD CAG size range and discussed their decision either not to have children or to do so only in the context of prenatal testing or preimplantation genetic diagnosis (PGD). One participant explained:

> *"The whole idea is to snip [HD] in the bud and if I'm going to have kids, I mean it's different now that there's prenatal testing but it's not business as usual if you get an intermediate."*

Another participant discussed deciding to refrain from having additional children but acknowledged both his wish to have PGD and the financial constraints that do not make this a feasible option:

> *"We were hoping to expand our family, we wanted to have more than one child but if Huntington's was a potential factor, we knew immediately that that's something we were going to stop and obviously finding out these results, we've opted not to extend our family and that's very difficult."*

Further, another individual spoke extensively about his journey to have a family. After a failed attempt at PGD, the couple underwent prenatal testing in a natural pregnancy and the fetus was found to have inherited two normal alleles ($\leq$26 CAG). The participant spoke of the ethical challenges they experienced, in particular deciding on the number of CAG repeats at which they would terminate the pregnancy and the possibility of receiving a mutation-positive PTR for their child if they decided not to terminate the pregnancy:

> *"Our decision changed as time went by. We were always in the mind that we would definitely keep it up until thirty-nine repeats but my mother was adamant that we should terminate if it was above thirty-six. [But] then we'd kind of looked at the sort of rough predictions [for] what age the disease happens with certain repeats and then we were going to keep it up to forty-one repeats but beyond [that repeat level] we were going to have a really serious decision to make."*

| Characteristics | n (%) |
|---|---|
| **Study Participants** | **29** |
| **Gender:** | |
| Female | 18 (62) |
| Male | 11 (38) |
| **Age (years)** | Average: 51.7, Range: 22-78 |
| **Marital Status:** | |
| Single | 6 (21) |
| Married | 21 (72) |
| Divorced | 2 (7) |
| **Children:** | |
| Have children | 22 (76) |
| No children | 7 (24) |
| **Education:** | |
| High School | 12 (41) |
| College | 7 (24) |
| Undergraduate | 7 (24) |
| Postgraduate | 3 (10) |
| **Employment:** | |
| Employed | 18 (62) |
| Retired | 7 (24) |
| Disability | 3 (10) |
| Homemaker | 1 (3) |
| **Time since predictive testing (years)** | Average: 9.7, Range: 1-16 |
| **Medical Genetics Service Providers** | **8** |
| **Position:** | |
| Medical Geneticist | 2 (25) |
| Genetic Counsellor | 5 (63) |
| Social Worker | 1 (13) |
| **Gender:** | |
| Female | 7 (88) |
| Male | 1 (13) |
| **Time providing predictive testing (years)** | Average: 10.4, Range 2-20 |

**Table 5.1 Demographic Characteristics of Study Participants and Medical Genetics Service Providers**

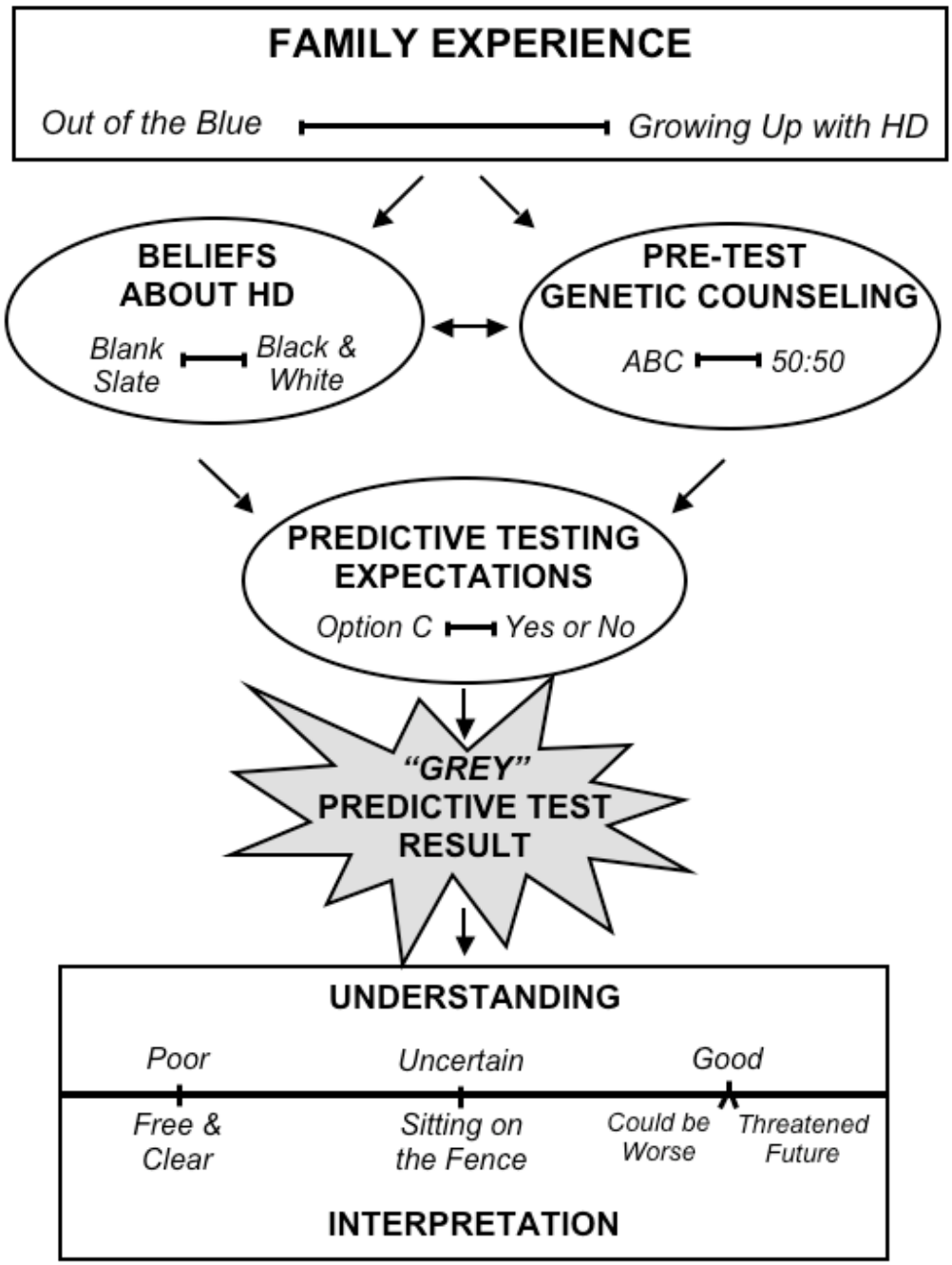



**Figure 5.1 The "*Grasping the Grey"* Theoretical Model**

| Category | n |
|---|---|
| **Total Number of Participants** | **29** |
| **Family Experience:** | |
| *Out of the Blue* | 14 |
| Sibling | 4 |
| Elderly Parent | 10 |
| *Growing Up with HD* | 15 |
| **Beliefs about HD:** | |
| *Blank Slate* | 12 |
| *Black & White* | 17 |
| **Pre-test Genetic Counselling:** | |
| *ABC* | 8 |
| *50:50* | 21 |
| **Predictive Testing Expectations:** | |
| *Option C* | 6 |
| *Yes or No* | 23 |
| **Understanding:** | |
| Poor | 6 |
| Uncertain | 8 |
| Good | 15 |
| **Interpretation:** | |
| *Free & Clear* | 6 |
| *Sitting on the Fence* | 8 |
| *Could be Worse* | 10 |
| *Threatened Future* | 5 |

**Table 5.2 Number of Study Participants in each Category of the "*Grasping the Grey*" Theoretical Model**

## 5.4 Discussion

The discovery of IAs has challenged beliefs about HD inheritance established almost 150 years ago and extends the risk of HD to the general population and families who have no history of the disorder. This is the first study to explore how individuals come to understand and interpret an IA-PTR. The *"Grasping the Grey"* theoretical model suggests that many individuals struggled to understand the clinical implications of an IA and had difficulty interpreting its significance for themselves and their family. Individuals' family experience, beliefs, pre-test genetic counselling, and predictive testing expectations influenced their understanding and interpretation of their IA-PTR. Many individuals either misunderstood, or were uncertain about, the clinical implications of their result. For individuals who had good understanding, many struggled with the uncertain risk of CAG repeat expansion causing a new mutation due to limited scientific knowledge.

Most striking in the "*Grasping the Grey"* theoretical model was the profound impact an individual's family experience had on their understanding and interpretation of an IA-PTR. Many studies have reported the powerful influence of family history on other aspects of the HD experience, including predictive testing decision making [Cox, 2003; Hamilton and Bowers, 2007] and risk perception [Cox and McKellin, 2001]. Given the hereditary nature of HD, there is the general perception that families affected with HD have a long-standing history of the disease. However, there is growing awareness that for some families, HD can be a new diagnosis, something never heard of in the family before. Similar to the work of Etchegary *et al.* [2006] and Forrest Keenan *et al.* [2007; 2009], individuals in this study described two different familial experiences: *"growing up with HD"* or experiencing HD *"out of the blue".* Individuals who grew up with HD had greater difficulty understanding an IA-PTR compared to those who experienced HD unexpectedly. Individuals with a long-standing family history have well-established *"black and white"* beliefs about the genetics of HD that conflict with IAs. These beliefs, together with pre-test genetic counselling, which focuses on the *"50:50"* inheritance pattern created *"yes or no"* expectations about predictive testing that were not met when they received an IA-

PTR. It is likely that the discordance between individuals' beliefs and expectations and the novel information on IAs acted as a barrier to their understanding.

How individuals come to understand and interpret uncertain genetic test results has received the most attention in the context of *BRCA 1* or *2* genetic testing for hereditary breast and ovarian cancer. Known *BRCA 1*/*2* mutations account for only 20-25% of familial breast and ovarian cancer cases [van Dijk *et al.*, 2006]. The majority of women receive *inconclusive results,* meaning that while a genetic alteration was identified, it is unclear whether or not it is a cancer-causing mutation or a benign change in DNA sequence. In some cases, testing family members can help clarify an uninformative result but in many cases uncertainty about the risk of cancer remains due to the limitations of current genetic technology and knowledge. Similar to the present study, Maheu [Maheu and Thorne, 2008] found that many women were shocked to receive an inconclusive *BRCA 1/2* result and had difficulty interpreting its meaning for themselves and their family based on personal beliefs and family experience. Hallowell [Hallowell *et al.*, 2002] found that some women misinterpreted their inconclusive result to mean that they either had a genetic mutation that significantly increased their cancer risk or that they did not have a mutation and, thus, their cancer risk was drastically decreased. Comparable results were found in our study where a proportion of individuals either interpreted their IA-PTR to mean they were still at-risk of the disease or that they and their family were free from the disease.

The amount of information that should be provided about IAs during genetic counselling for HD predictive testing has been debated [Maat-Kievit *et al.*, 2001b; van den Boer-van den Berg and Maat-Kievit, 2001] and concerns over inconsistencies in the information being provided between different testing centers have been raised [Tassicker *et al.*, 2006]. Interview data from both participants and medical genetics service providers suggested that there were no discrepancies in the information provided about IAs between the five predictive testing clinics. However, inconsistencies in the type of pre-test genetic counselling provided were

observed across participants and appeared to be influenced by family history. Only those individuals who had a sibling diagnosed with a new mutation received comprehensive pre-test information about IAs. This reflects an assumption within the medical genetics community that IAs are most often identified in families in which a new mutation has likely occurred. However, it is important to note that in this study, IAs were most often inherited from an unaffected parent on the non-affected side of an HD family. Data presented in Chapter 3, in addition to a study from Portugal [Sequeiros *et al.*, 2010], showed that 6% of individuals in the general population, with no known association to HD, had an IA. It is these 'general population' IAs on the non-affected side of an HD family that are often coincidentally ascertained in the context of genetic testing. While further studies on the frequency of IAs in different general populations are needed, this study suggests that approximately 1 in 17 individuals undergoing predictive testing may receive an IA from the non-HD side of their family. As such, comprehensive information on IAs should be provided to all individuals irrespective of their family history and future predictive testing guidelines need to standardize pre- and post-test genetic counselling practices related to IAs to ensure all individuals receive sufficient information and support.

This study highlighted the persuasive power of health beliefs and subsequent predictive testing expectations on the ability of participants to appreciate that IAs were a potential outcome of predictive testing. Despite all medical genetics service providers indicating that IAs were addressed in every pre-test counselling session when discussing the CAG repeat continuum, few participants specifically recalled this discussion. These findings suggest that equal emphasis is needed on all four possible PTRs (normal, intermediate, reduced, and full penetrance) and counselling needs to prepare individuals for results that do not conform to their expectations. Careful preparation of individuals in the pre-test counselling phase to all test outcomes, while time consuming, may help reduce feelings of shock and subsequent misunderstandings. Moreover, in order for individuals to make a fully informed decision about predictive testing, they must be aware of all result options. In addition to stressing the possibility of unforeseen results, pre-test counselling

should address the clinical implications of all PTRs and highlight the uncertainties in scientific knowledge. This is of particular importance given that many individuals' motivation for pursuing testing is to relieve uncertainty about the future [Bloch *et al.*, 1989; Decruyenaere *et al.*, 1995; Tibben *et al.*, 1993]. Individuals may also benefit from a discussion that explores their feelings about receiving a *"grey"* result that does not provide the certainty they may desire.

Given that the genetic and clinical implications of IAs are complex and uncertain, individuals who receive an IA-PTR likely have different education and support needs compared to persons who receive a mutation-positive or negative PTRs. Hallowell *et al.*, [2002] suggested that since the women in her study all received information on the clinical implication of an inconclusive *BRCA 1/2* result, their misunderstanding likely did not arise due to lack of information but instead the information may have been too complex for them to understand. It is possible that participants in the current study also struggled to understand the genetic complexity and uncertainty of IAs, particularly since the genetics of HD is largely perceived to be straightforward. Individuals' understanding of IAs may be improved with additional post-test genetic counselling to review the complex clinical implications and discuss the limitations of scientific knowledge. In particular, individuals with a long-standing family history may benefit from additional counselling as their engrained *"black and white"* beliefs and *"yes or no"* expectations may impede their ability to understand an IA-PTR. Additional post-test counselling would also provide an opportunity for genetic service providers to assess the level of understanding individuals have gained about IAs, identify misunderstandings, and provide additional information and support. This is particularly important for those individuals with poor understanding, who assumed that their family was *"free and clear"* and thus, were not motivated to pursue additional counselling.

The provision of written material describing the genetic and clinical aspects of an IAs would likely also benefit individuals' understanding, especially given that the information and resources available on IAs within the HD community are often vague

and can conflict with current scientific knowledge. As such, genetic counselling is likely one of the only sources of accurate knowledge on IAs. Individuals who receive an IA-PTR should also be encouraged to remain in contact with their medical genetics clinic and inquire about new knowledge and research on IAs. Studies that examine ways to present complex and uncertain genetic information, in both the context genetic counselling and the HD community, are also needed in order to communicate this information effectively and improve understanding.

The study findings highlight the degree of misunderstanding that exists within the HD community about IAs. While much of this uncertainty may be a result of the complexity of the information and its discrepancy from commonly held beliefs about HD, cognitive dissonance may also contribute to individuals' struggle to understand their *"grey"* PTR. Cognitive dissonance results in psychological discomfort when an individual perceives inconsistencies between their prior understanding and beliefs and new knowledge [Festinger, 1964; Grover, 2003]. Individuals who had more difficulty assimilating the novel information on IAs into their entrenched belief system may have subconsciously dismissed the meaning of their result in order to maintain their beliefs and relieve psychological stress. For example, in the breast cancer literature, van Dijk and colleagues [van Dijk *et al.*, 2005a; van Dijk *et al.*, 2005b] suggested that women who appeared to misinterpret the meaning of their inconclusive *BRCA 1/2* result may have been psychologically motivated to interpret their results incorrectly to cope with the associated clinical uncertainty. It is possible that individuals in this study, particularly those who grew up with the fear of HD, were using their misunderstanding or uncertainty as a coping strategy for dealing with the distressing and uncertain possibility of HD continuing in their family. More research is needed to explore the role of cognitive dissonance in how individuals come to understand and interpret uncertain genetic test results. Additionally, genetic counselling has to carefully balance an individual's need to protect themselves from psychological distress while ensuring they have the appropriate information to allow informed decision making.

The impact of an IA predictive test result on the psychological functioning of individuals with an IA for HD is unknown. This study suggests that these individuals experience a range of negative emotions including confusion, fear, guilt, anxiety and uncertainty. van Dijk [van Dijk *et al.*, 2008] demonstrated that while women who received an inconclusive *BRCA 1/2* test result do not report any adverse psychological consequences, their functioning was significantly worse than that of women who received a true negative result. Furthermore, women who reported feeling uncertain experienced higher levels of distress. These authors also showed that women who perceived themselves as having a high risk for a *BRCA 1/2* mutation based on a strong family history, had the greatest difficulty coming to terms with an inconclusive result [van Dijk *et al.*, 2006]. Collectively, these findings suggest that some individuals who receive an IA-PTR may experience increased psychological distress, particularly those participants who had a long-standing family history or were uncertain about the meaning of their *"grey"* result. Longitudinal research on the psychological impact of an IA is needed to identify potential psychological risk factors for adverse events after receiving an IA-PTR. An important caveat to future research on the psychological impact of an IA-PTR is the possibility that the distress experienced is a result of poor understanding about the clinical implications of an IA [Bish *et al.*, 2002].

Studies that examine how IA-PTRs are being communicated within families are also needed. While examining the familial communication process was not a specific aim of this study, the findings suggest that this process presents yet another challenge for individuals. Some participants discussed feeling unsupported by family members who discounted the clinical significance of their IA-PTR. Studies that explore the experience of disclosing an IA-PTR result may point to areas in which individuals can be supported in this communication process. Family counselling may be one way to support individuals in sharing IA-PTRs. In particular, offspring and extended family members on the non-HD side of the family, who have no knowledge of HD, may benefit from the education and support provided during genetic counselling. Educating family members about IAs provides an important opportunity to promote

awareness of this unique result and shift the predominant beliefs in the community about HD genetics to include IAs.

This study is not without some limitations. Firstly, participants in this study were a self-selected group with great diversity with regards to when they received their IA-PTR. Therefore, it is possible that the *"Grasping the Grey"* process does not reflect the collective experience of individuals and recall biases may have influenced the findings. However, there was a good representation amongst the different categories of the model, which suggests a range of experiences and perspectives were captured. Another limitation is that for participants who misunderstood their result, we were ethically unable to explore why they did not understand their result without informing them of their incorrect understanding. This weakness highlights the ethical challenge inherent to this research and raises questions about researchers' clinical responsibility to research participants who misunderstand the clinical implications of genetic test results. Lastly, this cross-sectional study examined understanding and interpretation at a single moment in time. Longitudinal studies are required to explore in more detail how understanding and interpretation of a *"grey"* result may shift over time.

The "*Grasping the Grey"* theoretical model adds to our limited knowledge on the experience of receiving IA-PTRs for HD. While more research is needed to examine how individuals come to understanding and interpret uncertain genetic information in other genetic diseases, the developed theoretical model may assist in ensuring this unique subset of individuals receive appropriate support, education, and genetic counselling during their predictive testing.

# Chapter 6: Discussion

## 6.1 Introduction

The clinical and molecular research conducted during this thesis has generated new knowledge on the frequency, haplotype, and CAG repeat instability of IAs for HD. The qualitative research has explored patient understanding and current genetic counselling practices regarding IA-PTRs. In summary, the familial transmission study showed 30% of IAs demonstrated intergenerational instability; of which 14% were CAG repeat expansions into the disease-associated range (≥36 CAG). The frequency and haplotype study revealed approximately 5.8% of individuals in B.C.'s general population, with no known association to HD, have an IA. Of the IAs ascertained in the general population, 60% are on a haplotype associated with a high-risk of CAG repeat instability. The sperm instability study established CAG-size specific risk estimates for IA repeat instability in paternal transmission and indicated that alleles at the upper limits of the intermediate CAG size range (34-35 CAG) have a significant risk (i.e. 2.5-21.0%) of expanding into the HD range (≥36 CAG). The qualitative interview study showed that genetic counselling practices regarding IA-PTR vary based on the individuals' family history and that individuals struggled to understand the clinical implications and significance of their IA-PTR. Collectively, these comprehensive findings increase our knowledge on the clinical significance of IAs and inform evidence-based genetic counselling implications regarding IA-PTRs.

## 6.2 Clinical Implications of Intermediate Alleles

At present, the clinical implication of IAs for HD is for offspring and/or future generations of the family to inherit an allele that expanded into the HD CAG size range (i.e. ≥36 CAG). The likelihood of CAG repeat expansion into the disease-associated range is highly influenced by the sex of the transmitting parent and CAG size. Consequently, the risk of a new mutation varies along a continuum of increasing magnitude (theoretical, low, moderate, and high risk) depending on these two factors (Figure 6.1).

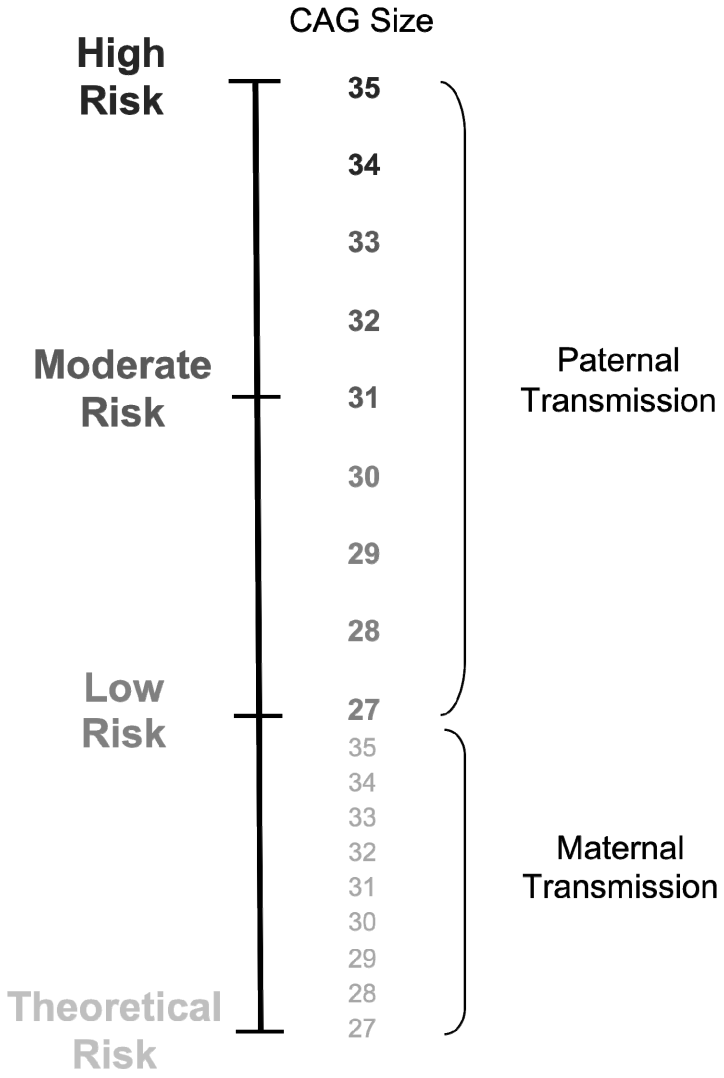



**Figure 6.1 Continuum of Risk for New Mutations Based on CAG Size and Sex of the Transmitting Parent**

The risk of a maternal new mutation due to CAG repeat expansion of IAs is largely theoretical. All documented new mutations for HD have occurred in paternal transmission. Only recently was there is a case report of maternal IA with 33 CAG repeat expanding into the disease range (48 CAG) [van Belzen *et al.*, 2009]. During this thesis, DNA from this maternal new mutation family was collected and haplotype analysis indicated that this maternal IA was not on the common haplotypes (i.e. A1 or A2) associated with a high-risk of CAG repeat instability. In fact, this maternal IA

was found on an unclassified haplotype that requires further study. Therefore, it is possible that unknown genetic or environmental modifiers are playing a role in this unusual case of a maternal IA CAG repeat expansion into the disease range. While maternal new mutation cases are extremely rare, CAG repeat expansion within the maternal germline has been documented. As reported in Chapter 2 (Table 2.2, page 56), 20% (n=17/86) of maternal IA transmissions in the UBC-HD database demonstrated CAG repeat instability. Of these unstable transmissions, approximately 41% (n=7/17) were repeat expansions, although not into the disease-associated range. As the number of maternal transmissions examined at each CAG size in the intermediate size range was exceedingly small (Table 2.3, page 57), the impact of CAG size on repeat instability in the maternal germline is not entirely clear and more research with larger sample sizes are needed. Therefore, while the risk that offspring of females with an IA will have a new mutation cannot be eliminated, especially for IAs at the upper limits of the intermediate CAG size range, it is extremely unlikely (Figure 6.1). Consequently, the clinical implications of maternal IAs are more relevant to future generations of the family, particularly if the IA is transmitted through the male germline.

Paternal transmissions of IAs are associated with the greatest empirical risk of new mutations. Data generated in the familial transmission (Chapter 2) and sperm (Chapter 4) studies showed alleles at every CAG size in the intermediate range (27-35 CAG) can expand into the HD range when passed through the male germline. The large sample size of the sperm study, both in terms of the number of IAs and sperm examined, has allowed for a more accurate quantification of the risk of CAG repeat expansion during paternal transmission. The data presented in Table 4.5 (page 105) demonstrates that the frequency of CAG repeat expansion $\geq$36 CAG dramatically increases over the intermediate CAG size range. Consequently, while there are clinical implications for all offspring of males with an IA, the significance of this risk is highly dependent on CAG size. Thus, paternal transmission of IAs are associated with three levels of risk (low, moderate, and high) for new mutations based on CAG size (Figure 6.1).

Paternal alleles with 34-35 CAG are associated with the highest risk for offspring to inherit a new mutation (Table 6.1). The sperm study showed that 2.4% (n=92/3850 sperm) of alleles with 34 CAG repeats (n=6) and 21.0% (n=481/2290 sperm) of alleles with 35 CAG repeats (n=4) expanded into the HD range (Table 4.5, page 105). When accounting for the transmission of one of two paternal alleles, this equals a risk for offspring to inherit an expanded allele with ≥36 CAG repeat of 1.20% and 10.50% for IAs with 34 and 35 CAG repeats, respectively. Offspring of fathers with a high-risk IA also have the greatest risk of inheriting a full penetrance HD allele (≥40 CAG) and consequently developing the classical HD phenotype. More specifically, offspring of males with 34 CAG have 0.15% risk to inherit an expanded allele ≥40 CAG and alleles with 35 CAG repeats have a 0.35% risk. IAs with 34-35 CAG are also associated with highest risk for offspring to inherit an expanded allele in the reduced penetrance range. The risk of transmitting an expanded allele with ≥36 CAG repeat is 1.05% and 10.15% for IAs with 34 and 35 CAG repeats, respectively. These offspring would likely experience a later age of disease onset, if they develop clinical features at all. For example, if an offspring inherited an expanded allele with 36 CAG repeats, the average age of onset would be approximately 66 years old [Langbehn *et al.*, 2004]. Moreover, only 29% of offspring with a 36 CAG allele would have a clinical phenotype by age 85. Table 6.2 reports the average age of symptom onset and penetrance rates of alleles in the 36-39 CAG size range [Langbehn *et al.*, 2004].

Paternal alleles with 31-33 CAG are associated with a moderate risk for offspring to inherit a new mutation (Table 6.1). The sperm study showed that 0.5% (n=7/1297sperm) of alleles with 31 CAG repeats (n=3) and 1.0% (n=16/1591 sperm) of alleles with 33 CAG repeats (n=2) expanded into the HD range (Table 4.5, page 105). This data suggests that the risk for offspring to inherit an expanded allele with ≥36 CAG for paternal transmission of IAs with 31–33 CAG ranges from 0.25% to 0.45%, respectively (Table 6.2). Offspring of fathers with a moderate–risk IAs have a relatively equal risk of expanded into the reduced (0.15–0.20%) or full penetrance (0.10–0.25%) range.

Paternal IAs in the low-risk category have between 27-30 CAG repeats and are associated with the lowest risk for offspring to inherit an expanded HD allele, ranging from 0.05–0.10% (Table 6.1). These risks are based on the sperm study, which showed that 0.1% (n=4/2907 sperm) of alleles with 27 CAG repeats (n=5) and 0.3% (n=6/2337 sperm) of alleles with 30 CAG repeats (n=4) expanded into the HD range (Table 4.5, page 105). Offspring of fathers with a low-risk IA are mostly likely to inherited an HD allele in the reduce penetrance range. In fact, no full penetrance HD expansions were observed until 30 CAG. IAs with 30 CAG repeats confer a 0.05% risk for offspring to inherit an expanded allele with ≥40 CAG. Given the low risks for offspring associated with paternal transmission of IAs with 27-30 CAG repeats, expansion into the HD range is mostly to occur in grandchildren and/or future generations of the family.

| Risk Category | CAG Size | Number of Alleles Examined | Risk for Offspring to Inherit an HD Allele (%) | | |
|---|---|---|---|---|---|
| | | | Total (≥36 CAG) | Reduced Penetrance (36-39 CAG) | Full Penetrance (≥40 CAG) |
| Low | 27 | 5 | 0.05 | 0.05 | 0.00 |
| | 28 | 3 | 0.05 | 0.05 | 0.00 |
| | 29 | 3 | 0.05 | 0.05 | 0.00 |
| | 30 | 4 | 0.15 | 0.10 | 0.05 |
| Moderate | 31 | 2 | 0.25 | 0.15 | 0.10 |
| | 32 | 1 | 0.25 | 0.00 | 0.25 |
| | 33 | 2 | 0.45 | 0.20 | 0.25 |
| High | 34 | 6 | 1.20 | 1.05 | 0.15 |
| | 35 | 4 | 10.50 | 10.15 | 0.35 |

**Table 6.1 Risk for Offspring to Inherit an HD Allele for Males with Low, Moderate, and High-Risk Intermediate Alleles**

| CAG Size | Average Age of Onset (years) | Penetrance Rates (%)* | | | |
|---|---|---|---|---|---|
| | | 70 years | 75 years | 80 years | 85 years |
| **36** | 66 | 0.10 | 0.14 | 0.21 | 0.29 |
| **37** | 55 | 0.17 | 0.26 | 0.37 | 0.49 |
| **38** | 64 | 0.32 | 0.45 | 0.60 | 0.73 |
| **39** | 58 | 0.53 | 0.69 | 0.81 | 0.90 |

* Pentrance rates reported as the percentage of individuals diagnosed with HD at given age
Adapted with permission from Langbehn et al., 2004

**Table 6.2 Average Age of Onset and Penetrance Rates for Alleles with 36-39 CAG Repeats**

## 6.3 Genetic Counselling Implications for Intermediate Alleles

The knowledge on IAs gained in this thesis has broadened our understanding of the clinical implications and significance of this unique PTR and has important implications for genetic counselling. Whether the IAs is associated with a theoretical, low, moderate, or high risk of new mutations can be used to inform genetic counselling practices. While the data generated in this thesis suggests all individuals undergoing predictive testing should receive comprehensive pre-test counselling on IAs, post-test counselling practices, such as the availability of prenatal testing, is dependent upon the magnitude of risk of repeat expansion into the HD range.

### 6.3.1 Pre-test Counselling

Collectively, the results of this thesis suggest that individuals undergoing predictive testing for HD would benefit from pre-test counselling that includes information on IA-PTRs. While it has been suggested that pre-test counselling on IAs may not be appropriate during the complex process of predictive testing decision making [Maat-Kievit *et al.*, 2001b], many individuals interviewed (Chapter 5) indicated that they wished they knew in advance that IA-PTRs were a possibility. The interview study also indicated that preparation for an IA-PTR may minimize feelings of shock and assist in long-term understanding of the clinical implications. Moreover, the frequency of IAs in the sample of B.C.'s general population, in addition to other

studies [Sequeiros *et al.*, 2010], also supports pre-test counselling on IA-PTRs given that 5.8% or approximately 1 in 17 persons undergoing testing will receive an IA-PTR. The relatively high likelihood of identifying an IA warrants education and preparation on all four possible PTRs, including normal, intermediate, reduced, and full penetrance PTRs during pre-test counselling.

The interview study (Chapter 5) revealed discrepancies in the pre-test counselling on IAs provided based on the individuals' family history. Individuals from new mutation families received the most pre-test information on IAs, whereas individuals with a long-standing family history received minimal knowledge. This counselling practice may reflect the belief that IAs are more likely to be identified in new mutation families and consequently, there is a greater need to educate and prepare clients for this PTR possibility. Contrary to this belief, however, this thesis demonstrated IAs are more often identified on the non-HD side of families with a long-standing history of the disease. The majority of participants in the sperm (87%, n=27/31) and interview (86%, n=25/29) studies had general population IAs inherited from their unaffected parent. A similar finding was observed in the clinical setting where the number of general population IAs (86%, n=116/135) in the UBC-HD Biobank exceeded new mutation IAs (14%, n=19/135). In fact, the Human Genetics Society of Australasia [2001] estimated that at least 2/3 of the time IAs are inherited from the non-affected side of an HD family. While no specific data was provided to support this claim, it reflects what was observed in this thesis. Consequently, all individuals, irrespective of their family history, should receive education and preparation on the possibility of an IA-PTRs in pre-test counselling.

Comprehensive pre-test counselling should highlight the possibility of receiving an IA-PTRs and describe the clinical implications for the individual, their children, and extended family members. The concept of CAG repeat instability and its association with the clinical implications could also be discussed, including factors associated with an increased risk of repeat expansion into the disease range. In families where a new mutation for HD has occurred, counselling could explain the relationship

between IAs and new mutations. Conversely, individuals with a long-standing history of HD could be prepared for the chance of unexpectedly inheriting an IA from the non-affected side of their family. Medical genetics professionals should emphasize the possibility of an IA-PTR as many individuals had never heard of IAs before, especially if they grew up with the disease in their family. Therefore, these individuals may lack the awareness and understanding that they could receive an IA-PTR. Pre-test counselling could also prepare individuals for a result that has clinical uncertainty given that many interview participants expressed an expectation that predictive testing would provide definitive information with clear clinical implications. Individuals may also benefit from a discussion that explores their desire to know a result with clinical uncertainty.

### 6.3.2 Risk Assessment for CAG Repeat Instability

CAG size and sex of the transmitting parent are the two factors that should be considered during clinical risk assessment of an IA-PTR. At present, the risk of new mutations for maternal transmission is primarily a theoretical risk; therefore, females who receive an IA-PTR can be reassured that the risk of their offspring receiving an expanded IA in the disease range is highly unlikely. Conversely, males who received an IA-PTR should be provided CAG-size specific risk estimates for their offspring to inherit an expanded allele in the HD CAG size range (Table 6.1). The magnitude of CAG repeat expansion is also important to considered during paternal risk assessment given that the majority (92.8%, n=566/610) of new mutations observed in the sperm study were within the reduced penetrance HD range (36-39 CAG). Consequently, while there is a risk that offspring may receive an expanded IA in the HD range, they may never display clinical manifestations or may have onset later in life. The risk for offspring to inherit an expanded allele with $\geq$36 CAG repeats should be considered in the context of hope that HD research will realize an effective therapy years prior to the offspring's symptom onset.

Interview data from medical genetic service providers (Chapter 5) indicated that the clinical context of the IA, whether the allele was ascertained in a new mutation family

or from the general population, is often used during risk assessment for CAG repeat expansion into the HD range. Service providers report being more reassuring about the risk of a new mutation when the IA is inherited from the non-affected side of the family. However, data generated in this thesis suggests that the IA's clinical context should not be a factor used in clinical risk assessment. While the familial transmission data did demonstrate a difference in instability between new mutation and general population IAs, it also showed that new mutation IAs had a significantly higher CAG size. Given that the sperm study produced strong evidence on the considerable impact of CAG size on the frequency of repeat instability, the disparity in rates of instability between these two categories is likely a reflection of their CAG size. Moreover, the haplotype study showed general population IAs have a high likelihood (60%, n=30/45) of being on a haplotype associated with a high-risk of CAG repeat instability. Despite no known association with HD, these general population IAs are expected to undergo CAG repeat expansion events over time, particularly when transmitted through the male germline. Therefore, the risk of CAG repeat expansion should not be minimized when an IA is ascertained from the general population.

While this thesis has generated data to inform clinical risk assessment of IA-PTRs, individuals could be cautioned that IAs represent a growing area of research in HD. The quantified CAG-size specific risk estimates based on the sperm study are relative risks of instability that do not account for unknown genetic or environmental factors that may influence the frequency and magnitude of instability. Therefore, while the numerical risk estimates are provided as a general assessment of repeat instability, individuals may have additional factors that may modify the risk of a new mutation. The relative nature of the risk figures could be highlighted, especially considering individuals may interpret these risks as having the same certainty as the risks associated with mutation-positive or negative PTRs.

### 6.3.3 Post-test Genetic Counselling

Individuals who are found to have an IA-PTR should be provided comprehensive post-test counselling on the clinical implications for themselves and their offspring. The magnitude of risk, based on the sex of the individual and CAG size, should also be clearly outlined. The concept of CAG repeat instability and its association with the clinical implications of an IA may also be reviewed in detail. The interview study (Chapter 5) showed that some individuals, especially those who were expecting definitive information, may struggle with feelings of confusion, uncertainty, or guilt regarding their IA-PTR. Therefore, these individuals may require further psychosocial support as they try to accept an unexpected result and grasp the unusual clinical implications. Individuals who have made plans to disclose their PTR to family members may also require additional post-test support as some participants interviewed indicated that they struggled with this communication process, particularly the challenge of informing family members of a PTR that is not well known.

All individuals would benefit from being provided with written material describing the genetic and clinical aspects of their IA-PTR during post-test counselling. Information and educational resources available on IAs within the HD community are often vague and can conflict with our current scientific understanding; therefore, these written materials would support individuals' long-term understanding and assist in family education. Through a collaborative effort with the Huntington Society of Canada, the knowledge generated in this thesis will be used to develop educational materials on IAs and new mutations for HD, which will enhance community understanding in the future. All individuals who receive an IA-PTR may also be invited to contact the clinic for additional education or support at any time in the future. Moreover, as scientific knowledge on IAs is expected to grow, individuals could be encouraged to stay in contact with the clinic and periodically inquire about new discoveries.

Given that the most significant risk of new mutations is associated with paternal transmissions of IAs with 34-35 CAG repeats, it is essential that these males have

accurate understanding of the clinical implications of their IA-PTRs. In fact, the interview study showed that many male participants had poor understanding about the clinical implications and some were not even aware of their misunderstanding. Therefore, males, particularly those with high-risk IAs, may benefit from additional follow-up counselling after result disclosure. This follow-up counselling will not only provide the opportunity for them to ask additional questions or request further support but also offers the chance for service providers to assess whether the men have any misunderstanding and review the relevant information, if required. Additional post-test counselling and education will likely improve understanding of the complex clinical implications of IAs, which is particularly important for males who hold well-established beliefs and expectations that may act as barriers to their understanding. Follow-up counselling could occur over the telephone after the individual has had sufficient time to reflect upon and absorb the new information on IA.

### 6.3.4 Prenatal Counselling and Testing

While prenatal counselling could be offered to all individuals who receive an IA-PTR, this counselling is of particular importance for males who have an IA with 34-35 CAG repeats. During prenatal counselling the clinical implications of an IA for offspring, the concept of CAG repeat instability, and the risk of a new mutation based on the individual's CAG size and sex, could be reviewed. During the interview study (Chapter 5), many individuals, including females, indicated that they would request prenatal counselling to clarify the clinical implications and magnitude of risk prior to starting a family. While prenatal counselling is of particular importance for males with a high-risk IA, it may also be relevant to females and males with smaller sized IAs, given that many of these individuals had poor or uncertain understanding. Prenatal counselling offers the opportunity to ensure individuals have accurate understanding upon which to base their reproductive decision-making.

While prenatal counselling could be available to individuals who receive an IA-PTR, prenatal testing should only be offered to couples who have a significant risk of CAG

repeat expansion into the HD range based on their sex and CAG size. The availability of prenatal testing should be based upon a balance between the risk of offspring inheriting an HD allele and pregnancy complications associated with the prenatal testing procedure, including chorionic villus sampling and amniocentesis. The CAG-size specific risk estimates for offspring to inherit an expanded HD allele suggests that males with 34-35 CAG repeats have a high-risk of producing a new mutation and thus should be eligible for prenatal testing. The risk of a new mutation associated with females and males with an IAs $\leq$33 CAG does not justify prenatal testing.

Couples eligible for prenatal testing should be engaged in a thorough discussion of the pros and cons of such testing and be encouraged to carefully weigh the likelihood of identifying an expanded allele in the HD range against the potential pregnancy complications as a result of the testing procedures. Despite the inherent risks associated with prenatal testing, the interview study indicated that individuals who have received an IA-PTR considered prenatal testing a feasible option. In fact, many male participants indicated they would only consider pursuing a family with the assistance of such testing.

While prenatal testing is a justifiable option for couples who have a considerable risk of a new mutation, the use of other reproductive technologies, such as preimplantation genetic diagnosis (PDG), is debatable. In general, PGD is offered to couples with a high risk of having offspring with a serious genetic disorder. Males with a 35 CAG are likely the most suitable candidates for PGD given that the offspring face a 10.5% risk of inheriting an expanded allele in the disease-associated range. Moreover, these offspring have the highest risk of inheriting a full penetrance HD allele. Nevertheless, couples interested in pursuing PGD could be engaged in a discussion that weighs the physical and psychological challenges and high monetary cost of PGD, against the likelihood of repeat expansion into the disease range. If this procedure is financially feasible, it may provide an acceptable alternative for couples

wishing to circumvent the possibility of pregnancy termination associated with traditional prenatal testing.

While the uptake of prenatal testing in the traditional context, when one parent has an allele in the disease-associated range, has generally been low [Adam *et al.*, 1993; Decruyenaere *et al.*, 2007], this prenatal testing scenario is also associated with a risk of identifying an IA. Based on the relatively high frequency of IAs in the general population, all cases of prenatal testing have a possibility of identifying an IA that was inherited from the non-HD side of the family. In fact, this situation occurred in the Netherlands when a couple, with one parent having an expanded HD allele with 43 CAG repeats, applied for prenatal diagnosis and the fetus was found to have an IA with 31 CAG repeats inherited from the unaffected parent [Maat-Kievit *et al.*, 2001b]. Consequently, couples pursuing prenatal testing for HD may benefit from a discussion on the possibility of unexpected results that may have uncertain clinical implications.

### 6.3.5 Genetic Counselling and Testing for Family Members

The responsibility of disseminating genetic risk information within a family lies with the tested individual. Familial risk communication is of particular importance in families found to have a high-risk IAs with 34-35 CAG repeats. Given that IAs are not well known in the general HD community and are associated with atypical clinical implications, tested individuals may require support in this communication process. Moreover, offspring and family members may request genetic counselling in order to clarify the unusual clinical implications of an IA for themselves. Family counselling sessions could be utilized to reduce the number, cost, and time of counselling sessions for offspring and family members of individuals found to have an IA. While genetic counselling is warranted for offspring and family members, only offspring of males with 34-35 CAG repeats should be eligible for predictive testing. Given that medical resources are limited, there must be an appropriate balance between the risk of a new mutation associated with the IA and the number of individuals eligible for predictive testing in a family found to have an IA.

Offspring eligible for genetic testing should be encouraged to post pone testing until they have fully adjusted to their new at-risk status. The interview study indicated that individuals who experienced HD 'out of the blue' were highly motivated to undergo predictive testing and did so almost immediately after learning of their at-risk status. A review of the predictive testing experience in Australia suggests a similar trend, where individuals who had limited familial exposure received predictive testing less than one year after finding out their at-risk status [Trembath *et al.*, 2006]. In light of this, medical genetic service providers should be aware that in the context of an IA, some persons may misjudge the impact and significance of predictive testing and their desire to quickly pursue predictive testing may simply reflect their limited knowledge and awareness of what it means to be at-risk. Consequently, these persons may benefit from delaying their genetic testing to allow them time to adjust to their new risk status and carefully consider their motivations and the potential ramifications of testing [Maat-Kievit *et al.*, 2001b; Trembath *et al.*, 2006].

## 6.4 Ethical Challenges

### 6.4.1 Duty to Recontact

The lower limits of the intermediate CAG size range have been redefined over the years as research has shown which CAG sizes can expand and produce new mutations [Goldberg *et al.*, 1995; Kelly *et al.*, 1999; Kremer *et al.*, 1994; Maat-Kievit *et al.*, 2001b]. Previous intermediate CAG size ranges were 30 to 35 [Kremer *et al.*, 1994] or 29 to 35 repeats [Goldberg *et al.*, 1995]. Consequently, there are persons who have an IA but never received counselling on the associated clinical implications. During participant recruitment for the interview study, there were numerous individuals with an IA who were not eligible to participate because they were not informed of the clinical implications of an IA. Such cases were also documented in the Netherlands, where individuals with IAs with less than 30 CAG repeats were not informed when the lower limit of the intermediate CAG size range was revised to include alleles with 27, 28, and 29 CAG repeats [Maat-Kievit *et al.*,

2001b]. These cases call into question our duty to recontact tested individuals with new information that modifies the clinical interpretation their PTR. It is current standard of practice for clinical services, particularly in medical genetics, to place the responsibility of maintaining in contact with the clinic on the patient or their primary care physician. This is justified by the large monetary and personnel costs it would be required if all individuals undergoing genetic testing had to be contacted when new information became available, especially given the rapid pace of advancing knowledge in medical genetics. However, when changes to the clinical interpretation of genetic test results are not a common occurrence, as in HD, this standard of practice could be questioned. While it has been suggested that clinicians may have a duty to re-contact individuals who were never counselled about their IAs [Maat-Kievit *et al.*, 2001b], the potential for introducing psychosocial distress must be weighed against a risk of expansion into the HD range that is substantially less than 1%, given that these IAs are at the lower limits of the intermediate CAG size range.

This thesis also highlighted the researcher's responsibility to their study participants. The interview study showed that many individuals did not have good understanding about the clinical implications of their IA-PTR. This finding emphasized the ethical tension between the researcher's role to document participant's understandings and the felt responsibility to improve participant's understandings. This challenge was especially salient when participants specifically asked the researcher to help them understand the clinical meaning of their PTR or when the researcher noted that the participant was using misinformation to inform reproductive decision making. While, in the current study, this tension was lessened by offering participants the opportunity for follow-up genetic counselling from their respective medical genetics clinic; this ethical challenge draws attention to the need for researchers to carefully consider the extent of their responsibilities when conducting clinical research.

### 6.4.2 Informed Consent

One of the guiding principles of genetic counselling is the need to acquire informed consent prior to genetic testing. The National Society of Genetic Counselors [1992]

Code of Ethics states that counsellors should *"enable their clients to make informed independent decisions… by providing or illuminating necessary facts*". Obtaining informed consent for predictive testing for HD is of the utmost importance – it is essential that the tested individual has a clear appreciation of the harms and benefits of testing and understands the implications and future consequences of testing for oneself and their family. In order for individuals to make an informed choice about predictive testing, they must be aware of the possibility of IA-PTRs and the clinical implications for themselves, their children, and extended family members. Given that IAs are not well known in the general community, combined with their clinical implications that contradicts common beliefs about the genetics of HD, it is essential that medical genetics service providers both educate and prepare individuals for this type of PTR during their pre-test genetic counselling. With the understanding that they may receive an unexpected PTR that has uncertain clinical implications, individuals can make a more informed decision about whether or not to pursue predictive testing.

As genetic testing rapidly expands and becomes more sophisticated, the number of unexpected genetic test results that have uncertain clinical implications will only increase. With improvements to molecular technologies and the advent of high throughput sequencing, private companies are now beginning to offer the general public the opportunity to have a wide array of genetic tests. These direct-to-consumer genetic testing services will increase the number of unexpected and uncertain genetic test results, including IAs for HD. Consequently, it may be time to examine our definition of informed consent and reach a consensus on what is acceptable in today's world of medical genetics and genomics. It is possible that standard informed consent should cover not only the issue of unexpected genetic test results but also results with uncertain clinical significance.

### 6.4.3 Prenatal Testing

Prenatal testing in the context of an IA also raises some important ethical questions. One challenge is in regards to the minimum CAG repeat length at which a couple

may choose to terminate the pregnancy. Given that the majority of expansions into the disease-associated range were within the reduced penetrance range (36-39 CAG), some couples may feel it is acceptable to continue a pregnancy with a reduced penetrance genotype. The late age of symptom onset associated with reduced penetrance alleles, combined with hope that progress in HD research will lead to future treatments, may make this a suitable option. In fact, during the interview study, one male participant with 35 CAG repeats spoke extensively about his experience with prenatal testing and the challenge of deciding on the number of CAG repeats at which the pregnancy would be terminated. This individual's medical genetics service provider also acknowledged this ethical challenge and expressed difficulty with the couples decision not to terminate a fetus found to have a reduced penetrance allele (36-39 CAG), as this circumstance could produce a mutation-positive PTR for a child, which is contrary to international best practice guidelines [IHA and WFN, 1994].

In light of this ethical challenge, couples who request prenatal testing in the context of a high-risk IA (males with 34-35 CAG repeats) should receive counselling on the harms associated with testing minors, including eliminating their child's right to make this decision as an adult and the potential for differential treatment, if they choose to complete a pregnancy after the fetus is found to have an expanded allele in the HD range. Fortunately, to our knowledge, this ethical challenge has not yet been realized. In the case of prenatal testing described in the interview study, the fetus was found to have a normal genotype. A similar outcome was also documented in the Netherlands when a female with 34 CAG underwent prenatal testing [Maat-Kievit *et al.*, 2001b]. Interestingly, a number of medical genetics service providers addressed this ethical challenge during the interview study and a common solution suggested was for the laboratory to only report the result as either in the HD range or not in order to avoid having definite knowledge of the fetus' genetic status. Panel discussions with scientists, clinicians, ethicists, and lay representatives are required to reach a consensus on this challenging ethical issue.

## 6.5 Future Research on Intermediate Alleles

### 6.5.1 Frequency of Intermediate Alleles

This thesis, in addition to recent studies [Ramos *et al.*, 2012; Sequeiros *et al.*, 2010], has provided preliminary data on the frequency of IAs for HD in the general population. Findings from these studies suggest the frequency of IAs in populations not associated with HD is relatively high and support the need for more detailed investigations. Additional frequency studies are needed that utilize larger sample sizes in diverse ethnic populations. Such studies will not only increase our knowledge on IAs but also shed further light on the origins and evolution of HD.

### 6.5.2 Maternal Intermediate Allele Repeat Instability

While the familial transmission data presented in this thesis adds to our knowledge on maternal CAG repeat instability, more data is required to establish empirical risk estimates and inform genetic counselling practices. Collaborative efforts are needed to increase the number of maternal IA transmissions examined and to increase the generalizability of empiric risk estimates. Further, clinicians could be encouraged to publish or present case reports on any occurrences of maternal new mutations in leading medical genetics journals and international conferences.

### 6.5.3 Psychosocial Impact of Intermediate Allele Predictive Test Results

The psychosocial impact and unique predictive testing experience of individuals who receive an IA-PTR requires further examination. The psychological functioning of individuals who receive an IA-PTR needs to be quantitatively measured using outcome measures such as depression and anxiety. Studies are also needed to establish the psychological functioning and quality of life of individuals before and after receiving their IA-PTR and make comparisons to individuals who receive mutation-positive or negative PTRs. This research may point to risk factors for adverse psychological response to an IA-PTR and further inform genetic counselling practices. Level of distress should be evaluated in the context of gender and family experience, as differences may exist between males and females and individuals

who have grown up with the disease or discovered it unexpectedly. The impact of the person's motivation for predictive testing, especially the desire to eliminate uncertainty, on psychological functioning should also be considered. We also need to more thoroughly understand individuals' risk perception regarding IA-PTRs and how it impacts their reproductive decision making. The communication process within families about IA-PTR is another area that requires further study. More specifically, when and how are individuals disclosing the implication of an IA-PTR to their offspring and extended family members and how is this risk information being perceived within the family?

### 6.5.4 New Areas of Uncertainty in Huntington Disease

New areas of uncertainties are arising in what was once thought to be a very straightforward genetic disease. Uncertainty in HD has primarily been examined in the context of living at-risk. Many individuals pursue predictive testing to relieve their uncertainty. However, predictive testing does not always provide straightforward clinical information. More specifically, individuals with a reduced penetrance HD allele face an uncertain risk to develop HD and persons with IAs must live with an uncertain future for their offspring and future generations. These unique categories of PTRs are expanding the meaning of genetic risk in HD. Yet surprisingly, the predictive testing and psychosocial experience of individuals who receive uncertain PTRs has not been widely explored. Further studies are needed to examine how individuals comprehend, cope, and adapt to uncertain genetic information and explore unique counselling and support needs.

Research is also needed to further examine patient and family understanding of uncertain PTR in HD, including intermediate and reduced penetrance alleles. It is essential that individuals have the appropriate understanding of the uncertainty aspects of their PTR in order to assist in informed decision making. Studies could examine how to effectively communicate uncertain PTR within the clinical setting and increase patient understanding. Community organizations could promote awareness of these new areas of uncertainty in HD and provide educational

opportunities that will help shift long-standing beliefs about the hereditary nature of HD to include new mutations. The impact of lay beliefs about genetics and inheritance on how individuals perceive uncertain PTRs in HD also warrants exploration.

### 6.5.5 Clinical Consequences of an Intermediate Allele for the Individual

The clinical implications of an IA for the individual is an emergent area of uncertainty in HD and it is possible a clinical phenotype could be defined in the future. Given that there have been a number of case reports documenting abnormal symptoms in the presence of IAs [Andrich *et al.*, 2008; Groen *et al.*, 2010; Ha and Jankovic, 2011; Herishanu *et al.*, 2009; Kenney *et al.*, 2007], research is urgently needed to clarify the clinical consequences of an IA for the individual. This research may include prospective studies that examine a large cohort of individuals with an IA for symptoms over time or retrospective case-control studies. Regardless, given the relatively high frequency of IAs in the general population, these studies will have to carefully exclude the possibility of a spurious association between clinical findings and intermediate CAG repeat lengths.

Since the discovery of the mutation underlying HD, a CAG length $\geq$36 repeats has been the gold standard to confirm a clinical diagnosis. This clear genetic criterion will continue to serve as a diagnostic requirement until there is sufficient evidence presented to support a change to the current CAG size ranges [ACMG and ASHG, 1998; Potter *et al.*, 2004]. The evidence required for a change should meet the same standards and rigor required when making a novel gene-disease association. The guidelines outlined in Table 6.3 may provide clinicians and scientists guidance on what evidence would be sufficient for a definitive HD diagnosis in the absence of $\geq$36 CAG repeats. These recommendations include clinical manifestations and specific neuropathological findings consistent with HD; exclusion of all disorders with clinical overlap to HD; and the demonstration of co-segregation of the IA with disease phenotype. Alterations to the CAG size ranges in HD would significantly change genetic counselling of at-risk individuals and their families, therapeutic trials, and our

current knowledge of the molecular pathogenesis of the disease. Therefore, caution must be taken when interpreting published case reports. The impact of erroneously altering the CAG repeat ranges would be detrimental to the both the scientific and general HD community. However, with evolving knowledge, it is possible that formal research studies will generate sufficient evidence to prove IAs confer clinical manifestations.

**Clinical Criteria:**

1. A phenotype consistent with HD including a *progressive* extrapryamidal movement disorder, including cognitive decline, and/or psychiatric disturbances
2. Exclusion of non-familial and familial disorders with clinical overlap to HD

   **Non-Familial:** Cerebral lupus, Chorea gravidarum, Tardive dyskinesia, Thyrotoxicosis, Sydenham chorea

   **Familial:** Ataxia-Telangiectasia, Autosomal Dominant Spinocerebellar ataxia – especially SCA17, Choreoacanthocytosis, Dentatorubral-Pallidoluysian atrophy, FAHR disease, Huntington disease-like 2, Huntington disease-like 1, Lesch-Nyhan syndrome, Neuroacanthocytosis, Pantothenate kinase-associated neurodegeneration, Wilson

**Neuropathological Criteria:**

1. Predominant neuronal cell loss in striatum by autopsy
2. Presence of huntingtin inclusions

**Evidence of heritability:**

1. Demonstration of autosomal dominant inheritance pattern
2. Demonstration of co-segregation of genetic change with disease

**Table 6.3 Guidelines for Diagnosing Huntington Disease with less than 36 CAG Repeats**

Phenotypic consequences of IAs or premutations in other trinucleotide disorders, including the spinocerebellar ataxias (SCAs), myotonic dystrophy, fragile X and Friedreich ataxia have been documented [Arsenault *et al.*, 2006; Gu *et al.*, 2004; Hagerman and Hagerman, 2002; Matilla-Dueñas *et al.*, 2008; Sharma *et al.*, 2004; Stevanin *et al.*, 1998; Yu *et al.*, 2011]. For example, the majority of women with fragile X premutations will experience premature ovarian failure and 20% will have cognitive impairments [Hagerman and Hagerman, 2002]. Older males with fragile X premutations have also been shown to develop a late onset fragile X tremor and ataxia syndrome [Hagerman and Hagerman, 2002]. Moreover, intermediate CAG repeat lengths in the ataxin-2 gene, responsible for SCA2, have been shown to be associated with the clinical phenotype of ALS, or Lou Gehrig's disease [Elden *et al.*, 2010]. Table 6.4 outlines additional phenotypic consequences of IAs in other triplet repeat disorders. Notably, the phenotypic effects of IAs in these trinucleotide repeat disorders were characterized years after the initial association between the classic disease phenotype and expanded repeat length were reported [Imbert *et al.*, 1996; Verkerk *et al.*, 1991]. Given the numerous similarities amongst the trinucleotide disorders, particularly the polyglutamine disorders like the SCAs, future research may show that IAs for HD also impart clinical consequences.

| Disease | Allele Category | Phenotype | Reference |
|---|---|---|---|
| **Spinocerebellar Ataxia (SCA) 1** | Intermediate [1] | Asymptomatic | (Matilla-Dueñas, 2008; Zühlke, 2002) |
| | Intermediate [2] | Mild clinical manifestations | |
| | Disease [2] | Progressive cerebellar ataxia, dysarthria, eventual deterioration of bulbar functions | |
| **SCA2** | Intermediate [1] | Risk loci for Amyotrophic lateral sclerosis (Lou Gehrig's disease) | (Elden, 2010; Yu, 201; Lu, 2004; Kim, 2007) |
| | Disease [1] | Parkinsonism | |
| | Disease [2] | Progressive cerebellar ataxia, including nystagmus, slow saccadic eye movements, ophthalmoparesis or parkinsonism | |
| **SCA3 (Machado-Joseph disease)** | Intermediate | Asymptomatic, atypical, or classic clinical manifestations | (van Schaik, 1997; Egan, 2000; van Alfen, 2001; Gu, 2004) |
| | Disease | Progressive cerebellar ataxia, dystonic-rigid syndrome, parkinsonian syndrome, or a combined syndrome of dystonia and peripheral neuropathy | |
| **Myotonic Dystrophy** | Premutation | Asymptomatic | (Yamagata, 1994; Arsenault, 2006) |
| | Protomutation | Asymptomatic or mild clinical manifestations (cataracts, mild myotonia) | |
| | Disease | Muscle weakness & wasting, myotonia, cataract, cardiac conduction abnormalities | |
| **Fragile X** | Intermediate: Female | Possible premature ovarian failure | (Hagerman, 2002; Aziz, 2003; Bennett, 2010; Madrigal, 2011; Chonchaiya, 2009; Willemsen, 2011) |
| | Intermediate: Male | Possible mild clinical manifestations (intellectual disability) | |
| | Premutation: Female | Possible emotional disturbances (depression, anxiety), premature ovarian failure, Fragile X-associated tremor and ataxia syndrome | |
| | Premutation: Male | Late onset Fragile X-associated tremor and ataxia syndrome | |
| | Disease: Female | Possible mild clinical manifestations (intellectual disability) | |
| | Disease: Male | Moderate intellectual disability, characteristic facial features, joint laxity, large testes after puberty, behavioral abnormalities | |
| **Friedreich Ataxia** | Premutation/Disease | Possible mild or classic clinical manifestations | (Sharma, 2004) |
| | Disease/Disease | Progressive ataxia, dysarthria, muscle weakness & spasticity in the lower limbs, scoliosis, bladder dysfunction, cardiomyopathy, diabetes | |
| **Dentatorubral-Pallidoluysian Atrophy (DRPLA)** | Intermediate: Homozygous | Spastic paraplegia | (Kurohara, 1997) |
| | Disease | Ataxia, choreoathetosis, dementia | |

**[1] Interrupted sequence; [2] Pure sequence**

**Table 6.4 Clinical Consequences of Intermediate Alleles in Other Trinucleotide Disorders**

As observed in the other trinucleotide diseases, the clinical consequences of IAs may be reminiscent of the traditional HD phenotype or, conversely, they could be unlike the characteristic disease features. In addition to the numerous case reports, two observational studies, published only in abstract form, summarize data on possible motor, cognitive, or behavioral abnormalities due to intermediate repeat lengths in HD. In the Prospective Huntington Disease At-Risk Observational Study (PHAROS), individuals with an IA were found to have similar motor, cognitive, and functional measures on the United Huntington Disease Rating Scale (UHDRS) compared to individuals with a control genotype; however, their behavioral scores were comparable to persons with an HD allele [Biglan *et al.*, 2010]. Further, significant differences in baseline UHDRS motor scores between individuals with a normal and intermediate genotype in the Cooperative Huntington's Observational Research Trial (COHORT) were identified [Ha *et al.*, 2011]. While these studies did not produce consistent findings, they suggest that IAs could produce a mild phenotype suggestive of traditional HD and highlight the need for further observational studies.

While this limited data suggests IAs for HD may confer clinical features, research is needed to explore the underlying pathological mechanism. It is possible that intermediate CAG repeat lengths fall at the end of the phenotypic spectrum in HD, such that they may confer a very late onset of symptom. Alternatively, individuals with an IA could display disease symptoms if they lived beyond our current lifespan. As inverse relationship between CAG size and age of onset is well recognized, it could be hypothesized that this correlation, which exists above the disease threshold of 36 CAG, also extends into the intermediate CAG size range. Moreover, the influence of unknown genetic or environmental modifiers on disease presentation has also been documented. The rare cases of a disease phenotype in the context of an IA may be due genetic or environment modifiers that accelerate the disease process resulting in earlier symptom onset [Groen *et al.*, 2010]. Indeed, it is possible that with the projected increase in our longevity, there may be an increase in the

number of persons with an IA who display a clinical phenotype [Tuljapurkar *et al.*, 2000].

Somatic instability may also contribute to an accelerated disease process in some individuals with an IA. In fact, somatic instability leading to large repeat expansions in the striatum and cerebral cortex of HD patients have been associated with earlier age of onset and more rapid disease progression [Swami *et al.*, 2009]. A single base excision repair enzyme called 7,8-dihydro-8-oxoguanine-DNA glycosylase (OGG1) has also been shown to be involved in progressive age-dependent somatic expansion in HD brains [Kovtun *et al.*, 2007]. Moreover, the neuronal population of the striatum was found to be particularly susceptible to a high rate of CAG repeat expansion, which is thought to enhance the toxicity of the mutant HTT protein [Gonitel *et al.*, 2008]. Collectively, this data suggests that tissue-specific differences in CAG length due to somatic instability could explain those individuals with an IA who display a clinical phenotype. In other words, while these individuals have a blood CAG size in the intermediate CAG size range, the CAG repeat tract in their striatal neurons may be above the disease threshold due to somatic instability.

While more research is needed, the published case reports may also represent unique instances were the pathogenicity of IAs is increased. Repeat lengths in the intermediate CAG size range have been shown to cause biochemical impairments. One study showed defective energy and metabolic impairments [Seong *et al.*, 2005] and another report suggested that individuals with an IA may have caudate glucose hypometabolism, which is impaired in presymptomatic individuals [Squitieri and Ciarmiello, 2010; Squitieri *et al.*, 2011]. Consequently, it is possible that some individuals with an IA may have a subtle phenotype due to subclinical HTT toxicity [Groen *et al.*, 2010; Squitieri *et al.*, 2011].

## 6.6 Conclusion

The unique combination of molecular and qualitative research contained in this thesis is the most substantial contribution to our knowledge on IAs for HD since they

were first described almost 20 years ago. IAs have changed the landscape of predictive testing for HD and challenge beliefs established over 150 years ago. While there is a multitude of psychological and social challenges that make the process of predictive testing difficult for individuals and their families, IAs have introduced additional complexity. The unexpected element of uncertainty in predictive testing is not only challenging for the tested individual, but also medical genetics service providers who struggle to interpret and communicate this clinical uncertainty.

While uncertainty is not uncommon in the field of medical genetics, the experience of receiving *"grey"* genetic test result will become increasingly more common as our scientific knowledge and technology advance, which will continue to present both ethical and clinical challenges. Moreover, as research gets closer to discovering a treatment for HD, more people will likely pursue predictive testing, and consequently there will be a growing number of persons who will receive an IA-PTR. Therefore, while the data presented in this thesis begins to fill numerous gaps in our scientific knowledge about IAs for HD, it is essential that we continue increasing our understanding, mostly importantly research is need to explore whether IAs impart clinical consequences for the individual.

It is hoped that the evidence-based genetic counselling implications outlined in this thesis will serve as the impetus to revise the current predictive testing guidelines so that IA-PTRs are appropriately acknowledged and addressed. Through consultation with an international panel of scientists, clinicians, lay organizations, and patients and families, formal predictive testing guidelines on this unique category of PTRs must be developed to represent best clinical practice. These guidelines will ensure individuals undergoing predictive testing receive standardized care and appropriate support, education, and counselling.

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# Appendix A

## A.1 Sperm Study Documentation: Letter of Invitation, Consent Form, Demographic Questionnaire, Donor Instructions, Thank you Letter

THE UNIVERSITY OF BRITISH COLUMBIA

**LETTER OF INVITATION**

**INTERGENERATIONAL CHANGES OF CAG SIZE IN THE HUNTINGTON DISEASE GENE**

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**[Date]**

Dear **[Donor Name]**,

We are writing to invite you to participate in an important study on Huntington disease (HD). This study is being led by principal investigator, Dr. Michael Hayden, at the University of British Columbia's Centre for Molecular Medicine and Therapeutics and his graduate student, Ms. Alicia Semaka.

In 1993, researchers identified the genetic mutation that causes HD. This genetic mutation involves the expansion of a small segment of our genetic material (DNA) within the HD gene. This segment of DNA in the HD gene is called a "CAG repeat". Everyone has a copy of the HD gene. It is the number of CAG repeats in the HD gene that determines if someone will eventually develop Huntington disease. Individuals that are affected with Huntington disease or those individuals found to carry the gene for Huntington disease through predictive testing have a higher number of CAG repeats in their HD gene compared to those individuals that will not develop Huntington disease.

You are being invited to participate in this study because you or one of your family members have had genetic (predictive) testing for Huntington disease.

Over the years, we have learned that the number of CAG repeats in the HD gene may change between generations for individuals in the general population. The CAG repeats may increase or decrease by a small number of repeats with no affect on the likelihood that an individual will develop Huntington disease. Alternately, the number of CAG repeats may remain the same.

The purpose of this study is to learn more about how the number of CAG repeats may change when the HD gene is passed on from parent to child. Another goal of this study is to identify specific factors that may determine whether the number of CAG repeats changes when passed from parent to child.

One way to study the changes in CAG repeats that may occur between parent and child is to study the number of CAG repeats in an individual's reproductive cells compared to the CAG repeat size in their blood cells. The CAG repeat size in an individual's reproductive cells would be identical to the CAG repeat size in that individual's child. The only reproductive cell that can be obtained with ease is sperm.

Participation in this study requires a semen (sperm) sample. There is no need to go to a clinic for the sperm donation. The sample can be collected at your home and mailed to the research lab. If you are interested in participating in this study, instructions for sending the sample to the lab will be sent to you once we receive a signed copy of the enclosed consent form.

There is no obligation to take part in this research study. If you do not wish to participate, please indicate this on the accompanying consent form and return using the enclosed reply envelope. The medical genetics care you receive will not be affected in anyway if you decline. Additionally, at any time after consenting to participate, you may withdraw from the study.

You will receive a monetary honorarium for participating in this study. You are free to accept this honorarium or if you prefer, you may donate it to the University of British Columbia's Centre for Molecular Medicine and Therapeutics to be used in future research on Huntington disease.

If you would like to be involved in this study, all information obtained will be kept strictly confidential. Your anonymity will be protected at all times by using a code number as an identifier and keeping all information in a secure location available only to members of the research team.

If you are willing to participate in this study, please sign and return the enclosed consent. Once your consent form is received, you will be sent an instruction sheet and collection materials to send your sample to the laboratory.

If you have any questions regarding the research, please feel free to contact Ms. Alicia Semaka at **(XXX) XXX-XXXX**. Thank you for your time and consideration.

Sincerely,

Michael R. Hayden, MB, ChB, PhD, FRCP(C), FRSC
University Killam Professor,
University of British Columbia, Department of Medical Genetics
Director and Senior Scientist,
Centre for Molecular Medicine and Therapeutics

Alicia Semaka, MSc, CCGC, CGC
Medical Genetics Doctoral Candidate
Genetic Counsellor
University of British Columbia
Centre for Molecular Medicine and Therapeutics

**SUBJECT INFORMATION AND CONSENT FORM**

**INTERGENERATIONAL CHANGES OF CAG REPEAT SIZE IN THE HUNTINGTON DISEASE GENE**

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**Principal Investigator:**
Dr. Michael Hayden
University Killam Professor
University of British Columbia
Department of Medical Genetics
Centre for Molecular Medicine and Therapeutics
**(XXX) XXX-XXXX**

**Co-Investigator:**
Alicia Semaka, MSc, CGC, CCGC
Medical Genetics Doctoral Candidate
Genetic Counsellor
University of British Columbia
Centre for Molecular Medicine and Therapeutics
**(XXX) XXX-XXXX**

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## WHAT IS THE PURPOSE OF THIS STUDY?

You are being invited to participate in a study of Huntington disease (HD). This study is being led by principal investigator, Dr. Michael Hayden, at the University of British Columbia and his graduate student, Ms. Alicia Semaka.

In 1993, researchers identified the genetic mutation that causes HD. This genetic mutation involves the expansion of a small segment of our genetic material (DNA) within the HD gene. This segment of DNA in the HD gene is called a “CAG repeat”. Everyone has copies of the HD gene. It is the number of CAG repeats in the HD gene that determines if someone will eventually develop Huntington disease. Most individuals in the general population have a small number of CAG repeats in their HD gene. Individuals who have a very high number of CAG repeats will be affected by HD.

You are being invited to participate in this study because you, or one of your family members, have undergone genetic testing for Huntington disease through the HD Clinic at UBC.

The number of CAG repeats in the HD gene may change between generations for individuals in the general population. Most often the number of CAG repeats may remain the same, but sometimes the CAG repeats may increase or decrease by a small number with no affect on the likelihood that an individual will develop Huntington disease.

The purpose of this study is to learn more about how the number of CAG repeats may change when the HD gene is passed on from parent to child and to identify specific factors that may determine whether the number of CAG repeats changes.

**WHAT DOES THIS STUDY INVOLVE?**

One way to study the changes in CAG repeats that may occur between parent and child is to study the number of CAG repeats in an individual's reproductive cells (i.e. sperm and eggs) compared to the CAG repeat size in their blood cells. The CAG repeat size in an individual's reproductive cells would be identical to the CAG repeat size seen in a child produced by fertilization of that reproductive cell.

The only reproductive cell that can be obtained with ease is sperm, which is present in semen produced by males. A semen sample contains millions of sperm, each of which could potentially result in a child if fertilization occurs.

Participation in this study requires a semen (sperm) sample. There is no need to go to a clinic for the sperm donation. The sample can be collected at your home and mailed to the research lab. If you decide to participate in this study, instructions for sending the sample to the lab will be sent to you once we receive a signed copy of the enclosed consent form.

Participation in this study also requires the completion of a short demographic questionnaire. The purpose of this questionnaire is to obtain some information about you, such as your age and your current health status. Completion of the questionnaire will take approximately 5-10 minutes. You do not have to answer any questions that you may feel uncomfortable answering.

**WHAT WILL HAPPEN TO YOUR SPERM SAMPLE?**

Your sperm sample will be sent to the Centre for Molecular Medicine and Therapeutics (CMMT) located in Vancouver. The CMMT is part of the University of British Columbia (UBC), Department of Medical Genetics. For the purpose of this research project, your genetic material (DNA) will be extracted from the sperm to analyze the number of CAG repeats in the HD gene.

Our hope is that the data derived from these samples will contribute to an ongoing program of HD research allowing us to explore new lines of investigation until a cure is found. With that in mind, all samples will be kept for an indefinite period of time, but used exclusively for this program of HD Research. Some samples may also be analyzed by scientific collaborators in other laboratories worldwide for the purposes of HD research. Any outside analysis will be performed in a completely anonymous manner using codes and will follow all protocols outlined in this research project.

**WHAT ARE THE RISKS AND BENEFITS OF THIS STUDY?**

There are no expected risks related to participating in this study. You will receive an honorarium of **$50.00** for participating in this study. You are free to accept this honorarium or if you prefer, you may donate it to UBC's CMMT to be used in future research on Huntington disease.

You will <u>not</u> receive any results from this study. The collected data from your sperm sample will only be used to help us better understand how the HD gene (number of CAG repeats) is passed from parent to child. Because any genetic testing ultimately performed on your sample will be experimental in nature, no individual results will be communicated to you or your family.

**WHAT ABOUT CONFIDENTIALITY?**

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 and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada and the UBC Research Ethics Boards 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.

All samples will be anonymously coded with unique identifiers and all information related to your samples will be kept confidential. All paper documents, such as your demographic questionnaire, will be stored in a locked file cabinet, available only to members of the research team. All computer and data files will be password protected. If you are ever seen as a patient at the UBC HD Medical Clinical in the future, your clinical data and contact information may be updated periodically in the database to ensure analysis using the most accurate data. Much of the information from this study may eventually be used in scientific publications, but your identity will not be revealed in any way.

**WHAT IF YOU HAVE QUESTIONS?**

We welcome any questions you may have about this study. If you have any questions at any time during your participation or you wish to withdraw your initial consent, please feel free to contact, Ms. Alicia Semaka at **(XXX) XXX-XXXX**. If you prefer, you may request to speak a male member of the research team at any time.

If you have any questions or concerns about your rights as a research subject and/or your experiences while participating in this study, please contact the Research Subject Information Line in the University of British Columbia's Office of Research Services, at **(XXX) XXX-XXXX**. This consent form is not a contract and as such you would not give up any legal rights by signing it.

**YOUR PARTICIPATION IS VOLUNTARY**

Your decision to donate a semen (sperm) sample and complete the accompanying demographic form is entirely voluntary. You may refuse or withdraw your consent and/or sample at any time. Upon notification that an individual has withdrawn from the study, all remaining samples will be destroyed; as well, all clinical and contact information in the database will be deleted. However, it may not always be possible to remove or delete research data results if they are no longer linked to an individual or have already been published in scientific articles. If you choose not to participate or withdraw, it will not affect your current or future medical care, or the care of any of your family members. You do not have to provide any reasons for your decision.

There is no obligation to take part in this research study. If you do not wish to participate or would like more information, simply call Ms. Alicia Semaka at **(XXX) XXX-XXXX**.

If you are willing to participate in this study, please sign and return the consent statement on the following page (Page 5) at your earliest convenience. Return the consent statement page using the enclosed stamped self-addressed envelope. Please keep the remaining pages of this document (Page 1-4) for your records.

THE UNIVERSITY OF BRITISH COLUMBIA

**CONSENT STATEMENT**

**INTERGENERATIONAL CHANGES OF CAG REPEAT SIZE IN THE HUNTINGTON DISEASE GENE**

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- My signature on this page indicates that I have read the above information and understand the risks, benefits, and procedures involved with participation in this study.
- I have had sufficient time to consider this information, ask questions and have received satisfactory responses.
- I understand that all of the information collected will be kept confidential and that all data and samples 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 at any time.
- I understand that I am not waiving any of my legal rights as a result of signing this consent form and I will be sent a dated signed copy of this form for my records.
- I voluntarily agree to donate a sperm sample for the purpose of Huntington disease research and complete the demographic questionnaire.

| | |
|---|---|
| **Donor Name (please print)** | **Telephone Number** |
| **Signature** | **Date** |
| **Witness Name (please print** | |
| **Signature** | **Date** |
| **Investigator Signature** | **Date** |

**If you have any questions regarding this consent form, please do not hesitate to contact Ms. Alicia Semaka at (XXX) XXX-XXXX**

THE UNIVERSITY OF BRITISH COLUMBIA

**DEMOGRAPHIC QUESTIONNAIRE**

**INTERGENERATIONAL CHANGES OF CAG REPEAT SIZE IN THE HUNTINGTON DISEASE GENE**

---

1.) Name: _____

2.) Date of Birth: _ _ / _ _ / _ _ _ _
DD/ MM / YEAR

3.) Date sperm sample was collected: _ _ / _ _ / _ _ _ _
DD/ MM / YEAR

b.) Approximately what time did you collect the sample?

c.) Were you able to collect a complete sperm sample?

☐ Yes ☐ No

d.) If you were <u>not</u> able to collect the complete sperm sample, please indicate which half was collected?

☐ Most of the first half of the sample

☐ Most of the last half of the sample

☐ Other: Please Specify:

4.) What is the ethnic background of the following family members i.e. Indo-Canadian, Caucasian (white/British/European), Asian (Chinese/Japanese/Korean/Vietnamese):

Paternal Grandfather: _____ (i.e. Father's Father)

Paternal Grandmother: _____ (i.e. Father's Mother)

Maternal Grandfather: _____ (i.e. Mother's Father)

Maternal Grandmother: _____ (i.e. Mother's Mother)

5a.) Do you currently have any medical conditions?

☐ Yes ☐ No

b) If yes, please specify: _____

6a.) Have you previously had any medical conditions?

☐ Yes ☐ No

b.) If yes, please specify: _____

7a.) Are you currently take any prescription medication?

☐ Yes ☐ No

b.) If yes, please specify name of medication, dose, quantity and reason for taking medication in following table

| | **Medication Name:** | **Dose:** | **Quantity:** | **Reason**: |
|---|---|---|---|---|
| i.e. | Tetrabenazine | 25mg | 3 X daily | Chorea |
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |

8a.) Are you currently take any vitamins and/or minerals?

☐ Yes ☐ No

b.) If yes, please specify name of vitamin and/or, dose, quantity and reason for taking vitamin and/or mineral in following table

| | **Vitamin Name:** | **Dose:** | **Quantity:** | **Reason**: |
|---|---|---|---|---|
| i.e. | Calcium | 50 mg | Once daily | General health |
| 1. | | | | |
| 2. | | | | |
| 3. | | | | |

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**INSTRUCTIONS FOR SAMPLE COLLECTION AND SHIPMENT**

**INTERGENERATIONAL CHANGES OF CAG REPEAT SIZE IN THE HUNTINGTON DISEASE GENE**

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The following document contains the semen sample collection and shipping instructions. Please read and follow these guidelines carefully. If you have any questions or concerns about this research study or these instructions, please call Ms. Alicia Semaka at **(XXX) XXX-XXXX** .

We realize semen collection can be embarrassing and some individuals may want to modify the collection procedure. However, sperm cells are very delicate and any deviation from these instructions may compromise the quality of the sample and research results.

This package should contain:

- One styrofoam shipping chest in cardboard box
- One clear plastic sample collection cup sealed in plastic wrapping
- One clear reclosable “biohazard” bag
- One “polar pack” foam brick ice pack
- One sticky label with your unique identification code
- A handful of styrofoam peanuts
- A demographic questionnaire
- A requisition for return shipping
- 3 copies of the commercial invoice for Canadian Customs (if applicable)

**OPTIONS FOR SAMPLE SHIPMENT:**

**[Name of Shipping Company]** will be used for sample shipment. All shipping costs have been prepaid. There are two different options for sample shipment. Please choose the option that will be most convenient for you.

1. The sample can be picked up directly from your home and shipped to the research laboratory. To arrange sample pick up please call **[Telephone Number]**

2. You may drop off the sample yourself at a local drop off center before 12:00pm. A drop off location near your house is listed below although you may also drop off the sample at another location that may be more convenient for you.

**[Address]**
**[Telephone Number]**

**INSTRUCTIONS FOR SAMPLE SHIPPMENT:**

1. The sample needs to be collected and shipped to the research laboratory in a timely manner.

A. The sample should be collected in the morning of the same day that it will be shipped.

B. The sample should be shipped on a Monday, Tuesday, or Wednesday only. This is to ensure the sample does not arrive on a weekend when no one will be at the research laboratory to receive it.

2. 24 hours prior to sample collection and shipment, please place the “polar pack” foam brick ice pack in your freezer. Please have tape to seal the cardboard shipping box on hand.

**INSTRUCTIONS FOR SEMEN COLLECTION:**

1. You should abstain from any sexual activity, including masturbation for a minimum of 2 days prior to collection of the semen sample. Ideally, it should be more than 2 days from a previous ejaculation and not more than 7 to 10 days. This will help improve the quality of the sample.

2. Prior to collecting the sample, washed your hands and penis with soap and water. Dry thoroughly.

3. After you have washed your hands, remove the clear plastic collection cup from its plastic wrapping. Stick the enclosed label with your unique identification code onto the side of the collection cup. Unscrew the collection cup lid.

4. The sample should be collected by masturbation directly into the cup.

> A. Lubricants should not be used to aid in the collection of the sperm as they may be toxic to sperm or interfere with the molecular techniques used during the sperm analysis.
>
> B. Interrupted intercourse should not be performed for specimen collection as this may results in the loss of the most critical portion of the ejaculate (pre-ejaculate) and the specimen may be contaminated with cells or bacteria from the vagina.
>
> C. If a pubic hair or thread of clothing accidentally falls into the container, do not attempt to remove it, the lab will remove it using sterile techniques.

5. After collection of the semen in the cup, screw on the lid tightly and place it in the clear Biohazard bag. Squeeze the bag to remove any extra air and seal the bag closed.

6. Please complete the demographic questionnaire. If a portion of the sample was lost during collection, please indicate on this form. Place the demographic questionnaire into the separate side pocket of the plastic biohazard bag.

7. Retrieve the “polar pack” foam brick ice pack from your freezer and place it in the bottom of the Styrofoam Shipping Chest. Place the bagged sample on top of the foam brick and fill the surrounding area with the styrofoam peanuts so the cup does not shift during transport.

8. Replace the lid of the styrofoam shipping Chest and seal the cardboard box with tape.

9. Remove the shipping requisition that was used to send you the collection kit from the plastic envelope and replace it with the enclosed return shipping requisition. The majority of this requisition has been filled in for you, just insert the date that the sample will be shipped and your signature (highlighted area).

A. If you live outside Canada, please include the three copies of the commercial invoice in the plastic envelope.  he majority of this invoice has been filled in for you, just add the date that the sample will be shipped and your signature (highlighted area) to each copy of the invoice.

10. Please telephone Ms. Semaka at **(XXX) XXX-XXXX** to inform her that you have shipped your sample. Please leave a message if there is no answer.

**Once again, thank you for your participation in this research study.  Your contribution to this study is appreciated and valued.  We will confirm receipt of your sample by mail contact once it is received.**

THE UNIVERSITY OF BRITISH COLUMBIA

**THANK YOU LETTER**

**INTERGENERATIONAL CHANGES OF CAG REPEAT SIZE IN THE HUNTINGTON DISEASE GENE**

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**[Date]**

Dear **[Donor Name]**

This letter is to inform you we recently received your sperm donation for the above named study.

We would also like to extend our sincerest thank you for your willingness to participate in this study. We believe that exceptional science starts with exceptional study participants. Your sperm donation will go far in helping us to understand how the number of CAG repeats in the HD gene may change when passed from parent to child.

Please accept the enclosed $50.00 honorarium for your participation. You are free to accept this honorarium or if you prefer, you may donate it to the University of British Columbia Centre for Molecular Medicine & Therapeutics to be used in future research on Huntington disease.

If you have any further questions regarding this study or you would like to be informed when the results of this study are published, please feel free to contact Ms. Alicia Semaka at (XXX) XXX-XXXX. Once again, we value and appreciate your contribution,

With Gratitude,

Michael R. Hayden, MB, ChB, PhD, FRCP(C), FRSC
University Killam Professor,
University of British Columbia, Department of Medical Genetics
Director and Senior Scientist,
Centre for Molecular Medicine and Therapeutics

Alicia Semaka, MSc, CCGC, CGC
Medical Genetics Doctoral Candidate
Genetic Counsellor
University of British Columbia
Centre for Molecular Medicine and Therapeutics

## A.2 Interview Study Documentation: Participant and Medical Genetics Service Provider Letter of Invitation, Consent Form, Interview Guides

THE UNIVERSITY OF BRITISH COLUMBIA


**PARTICIPANT LETTER OF INVITATION**

**DEVELOPMENT OF HUNTINGTON DISEASE PREDICTIVE TESTING GUIDLINES**

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**[Date]**

Dear **[Participant Name]**

I am writing to invite you to participate in an important study because you have undergone genetic (predictive) testing for Huntington disease. This study is being led by principal investigator, Dr. Michael Hayden, at the University of British Columbia and his graduate student, Ms. Alicia Semaka.

The purpose of this study is to learn more about the experience of individuals who have received predictive-test results similar to the type of results you received. Currently, there is no information in the scientific literature about the individual and family experience of people who have received this type of predictive-test result.

In the years since predictive (genetic) testing first became available, we have learned that some predictive-test results are very complex and difficult to understand and explain to other family members. Through this research, we hope to learn how to better support, educate, and counsel individuals who undergo predictive (genetic) testing for HD and receive predictive-test results like the ones you received. Additionally, this study may emphasize the need for an improved genetic counselling protocol for Huntington disease, which specifically addresses the needs of individuals found to have this type of predictive-test result.

Participation in this study involves an interview, which will take approximately one hour to complete. The interview will ideally take place in-person, either at your home or at an alternative location, such as the Medical Genetics Clinic in your area. If an in-person interview is not convenient, the interview can be conducted over the telephone. The interview will largely consist of questions regarding your predictive (genetic) testing experience. The interview will also explore your thoughts, feelings, and perceptions about your specific predictive-test result.

There is no obligation to take part in this research study. If you do not wish to participate, please indicate this on the accompanying consent form and return it using the enclosed stamped, self-addressed reply envelope. The care that you receive will not be affected in anyway if you decline. Additionally, at any time after consenting to participate, you may withdraw from the study without consequence.

If you would like to participate in this study, all information obtained will be kept strictly confidential. Your anonymity will be protected at all times by using a code number as an identifier and keeping all information in a secure location available only to members of the research team.

If you are willing to participate in this study, please sign and return the enclosed consent form. A pre-addressed, stamped envelope is included. Upon receiving your consent form, you will be contacted to arrange a time, date, and location, which will be convenient for you to privately participate in the interview.

If you have any questions regarding the research, please feel free to contact Ms. Alicia Semaka at **(XXX) XXX XXXX**. Thank you for your time and consideration.

Sincerely,

**[Name and Credentials of Physician and Genetic Counsellor]**

THE UNIVERSITY OF BRITISH COLUMBIA

**PARTICIPANT CONSENT FORM**

**DEVELOPMENT OF HUNTINGTON DISEASE PREDICTIVE TESTING GUIDELINES**

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**Principal Investigator:**

Dr. Michael Hayden
University Killam Professor
University of British Columbia
Department of Medical Genetics
Centre for Molecular Medicine and Therapeutics
**(XXX) XXX XXXX**

**Co Investigators:**

Alicia Semaka, MSc
Medical Genetics Doctoral Student
University of British Columbia
Department of Medical Genetics
Centre for Molecular Medicine and Therapeutics
**(XXX) XXX XXXX**

Dr. Lynda Balneaves
Assistant Professor
University of British Columbia
School of Nursing
**(XXX) XXX XXXX**

---

## WHAT IS THE PURPOSE OF THIS STUDY?

We are inviting you to participate in an important study because you have undergone predictive (genetic) testing for Huntington disease. The purpose of this study is to learn more about the experience of individuals who have received predictive-test results similar to the results you received. This research is being performed as a requirement of a postgraduate degree in Medical Genetics and the results of this study will reported in the student's dissertation.

Currently, there is no information in the scientific literature about the individual and family experience of people who have received this type of predictive-test result. In the years since predictive (genetic) testing first became available, we have learned that some predictive-test results are very complex and difficult to understand and explain to other family members. Through this research, we hope to learn how to better support, educate, and counsel individuals who undergo predictive (genetic) testing for Huntington disease and receive results like the ones you received. It is anticipated that the information gathered from this study will help improve genetic counselling for individuals and families at risk for Huntington disease. Additionally, this study may emphasize the need for an improved genetic counselling protocol for Huntington disease, which specifically addresses the needs of individuals found to have this type of predictive-test result.

## WHAT DOES THIS STUDY INVOLVE?

Participation in this study involves an interview, which will take approximately one hour to complete. The interview will ideally take place in-person, either at your home or at an alternative location, such

as the Medical Genetics Clinic in your area. If an in-person interview is not convenient, the interview can be conducted over the telephone.

The interview will largely consist of questions regarding your predictive (genetic) testing experience and will explore your thoughts, feelings, and perceptions about your specific predictive-test result. The interview questions will be open-ended, in order to allow you to speak freely, and share as much, or as little as you feel comfortable in doing.

With your permission, we would like to audiotape the interview in order to transcribe the conversation for analysis. If you are not comfortable with this, the interview will not be recorded. Furthermore, if you would like the tape recording to be stopped at any time during the questionnaire, this will be arranged.

You may be contacted to participate in a follow-up interview. The purpose of a second interview would be to clarify anything discussed in the first interview, ask you some additional questions, and/or share the results of this study with you for your opinion. A follow–up interview will take approximately ½ hour and will be tape-recorded. This interview may be conducted in-person or over the telephone. Involvement in a second interview is not required for participation in this study.

**HOW DO YOU BECOME INVOLVED IN THIS STUDY?**

If you wish to participate in this study, please sign and return this consent form using the enclosed self-addressed, stamped envelope by **[DATE]**. Once we have received your consent form, you will be contacted to arrange a time, date, and location that will be convenient for you to participate in the interview.

There is no obligation to take part in this research study. If you do not wish to participate, please indicate this on the consent form and return the form using the self-addressed, stamped envelope. The care that you receive will not be affected in anyway if you decline. Additionally, at any time after consenting to participate, you are free to withdraw from this study.

**WHAT ARE THE RISKS AND BENEFITS OF THIS STUDY?**

You will not receive any direct benefit from taking part in this study. However, we think the results of this study will help improve predictive (genetic) testing for Huntington disease for individuals who receive the same type of predictive-test result you received.

There are no expected risks related to participating in this study. However, it is possible you will find the nature of the topics addressed in this interview upsetting. If these feelings occur, with your permission, the genetic counsellor working with this research team will contact you to discuss any concerns or issues you may have experienced during your participation in this study and direct you to additional support, as needed. You will also be provided with the contact information for your Medical Genetics Clinic, should you have questions or concerns at a later date.

**WHAT ABOUT CONFIDENTIALITY?**

Your participation in this study will be kept confidential to the extent permitted by law. Your anonymity will be protected at all times by using a code number as an identifier and keeping all information in a locked file cabinet, available only to members of the research team. All computer files will be password protected. When transcribing the audiotaped interview, all names and any identifying information will be removed.

The interview transcripts and audiotapes will be kept for the duration of 5 years, in compliance with University of British Columbia research policy. After this time, they will be destroyed in a manner that will ensure confidentiality. In the event of any report or publication from this research, the identity of the participants will not be revealed and the results will be summarized in a manner that participants cannot be identified.

**WHAT IF YOU HAVE QUESTIONS?**

We welcome any questions you may have about this study. If you have any questions or you wish to withdraw your initial consent, please feel free to contact the study coordinator, Ms. Alicia Semaka at **(XXX) XXX XXXX**.

If you have any questions or concerns about your treatment or rights as a research subject, please contact the Research Subject Information Line in the University of British Columbia's Office of Research Services, at **(XXX) XXX XXXX**.

**********

Your signature below indicated that you have read the above information, understand the risks, benefits, and procedures of the study, and voluntarily agree to participate in this research project.

Please keep one copy of this consent form for your records and return the other copy of the consent form using the self addressed, stamped envelope included by **[DATE]**.

| | |
|---|---|
| _____ | _____ |
| Participant Name | Telephone Number |
| _____ | _____ |
| Signature | Date |

THE UNIVERSITY OF BRITISH COLUMBIA

**PARTICIPANT INTERVIEW GUIDE**

**DEVELOPMENT OF HUNTINGTON DISEASE PREDICTIVE TESTING GUIDELINES**

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**Date:**

**Time:**

**Location:**

**Interviewee Name:**

**Interview Code Number:**

*"Thank you for agreeing to participate in this interview. My name is Alicia Semaka. I am a medical genetics doctoral student. This research is being performed as part of my dissertation. Once again, the purpose of this study is to learn more about the predictive (genetic) testing experience of individuals who have received predictive-test results like your own."*

*"If for any reason you no longer wish to participate in this study, you are under no obligation and can do so with out consequence. Would you like to proceed with the interview?"*

*"Please remember that if you would like to stop the interview at anytime or would like to take a break, you are free to do so. We can always schedule an alternate time to complete the interview, if you wish."*

*"In addition, if you feel any distress at all during the interview, you are free to stop your participation. If these feelings occur, with your permission, I will have the genetic counsellor working with this research team contact you to further assist you and direct you to additional support as needed. You will also be provided with the contact information for your local medical genetics clinic should you have questions or concerns at a later date."*

*"Just a reminder, I would like to audio tape this interview in order to transcribe our conversation for easier analysis. If you are not comfortable with this, please let me know at this point and our conversation will not be recorded. Furthermore, if you would like me to turn off the recorder at any point during the interview, just let me know."*

*"Lastly, please keep in mind that everything you say during this interview will be kept strictly confidential and will only be shared with members of the research team. Your name and all identifying information will be removed. Your interview transcript will receive a code number that will not identify you in anyways. After the interview is transcribed, all audiotapes will be destroyed. All sensitive material obtained during this research study will be stored in a secure location."*

*"This interview will take approximately one hour to complete. I will be using an interview guide to help ensure all topic areas are discussed in each interview. The questions in this interview are open-ended, so please speak freely, and share as much, or as little as you feel comfortable in doing. Please feel free to tell me if you would prefer not to answer a question that I ask. Furthermore, if you have any questions during the interview, please ask."*

*"With your permission, I may ask you speak to you again at a later date. The purpose of a second interview would be to clarify anything discussed today, ask you some additional questions, and/or share the results of this study with you for your opinion."*

*"Before we begin, do you have any questions?"*

*"To start, I would first like to get some demographic and family history information from you."*

---

**DEMOGRAPHIC INFORMATION**

1.) Gender:

☐ Male ☐ Female

2.) DOB: _ _ / _ _ / _ _ _ _
(MM / DD / YEAR)

3.) Martial Status:

☐ Single ☐ Married ☐ Divorced

☐ Separated ☐ Common Law ☐ Widow

4.) What cultural or ethnic group do you most closely associate with?

5a.) Are there any medical conditions you are living with?

☐ Yes ☐ No

5b.) If yes, please specify:

6a.) Do you have any children?

☐ Yes ☐ No

7b.) DOB and Sex of Children:

1.
2.
3.
4.
5.

8.) Highest Level of Formal Education:

9.) Occupation:

10.) Currently employed:

☐ Yes ☐ No

11.) Year found out HD in family:

12.) Year underwent predictive (genetic) testing for HD:

13.) City in which you underwent predictive (genetic) testing for HD:

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**FAMILY HISTORY**

**Pedigree:** Ask about individuals affected with HD including their DOB, age of onset, and if applicable, their age of death. Ask about individuals who have undergone predictive (genetic) testing including result, year of testing, age at testing, and current age.

## PARTICIPANT INTERVIEW GUIDE #1

**1.) To get us started, can you tell me how you have been since your predictive (genetic) testing?**

**2.) Please tell me how you came to pursue predictive (genetic) testing for Huntington disease?**

- What was going on in your life prior to deciding to undergo predictive (genetic) testing for Huntington disease?

**3.) Please tell me what it was like to go through the process of predictive (genetic) testing (i.e. from going in for your first genetic counselling session, to receiving your predictive-test results)?**

**4.) What, if anything, did you know about the possible predictive-test results, prior to actually receiving your result?**

**5.) Please tell me what you recall being told about your predictive-test result?**

- Do you recall what your predictive-test result was called?
- What was the term the medical geneticists/genetic counsellor used?]

**6.) How do you feel you understand the implications of your predictive-test result for yourself, your children, and your extended family members?**

- What do you understand about your predictive-test result for yourself, your children, and your extended family members?
- What do you not understand about your predictive-test result for yourself, your children, and your extended family members?
- How do you think your understanding of your predictive-test result could be improved?

**7.) How did you feel about your predictive-test result initially?**

- How do you feel about your predictive-test result now?
-

**8.) Did you discuss your predictive-test result with anyone?**

- With whom did you discuss your predictive-test result?
- How did you choose to discuss your predictive-test result with above?
- What did you discuss?
- What was their reaction to what you discussed?
- With whom did you decide not to discuss your predictive-test result?
- How did you choose not to discuss your predictive-test result with this person/these people?

**9.) How has your predictive-test result influenced your life, if at all?**

- How has your predictive-test result influenced your family relationships, if at all?
- How has your predictive-test result influenced your future plans (i.e. life plans, career plans, family, plans, and financial plans)?
- What have you done, or not done, because of your predictive-test result?
- How has your life changed since receiving your predictive-test result? Do you consider these positive or negative changes?

**10.) Have you ever been treated differently (discriminated against) by your friends, family, work colleagues, because of your predictive-test result?**

- Can you tell me about this?

**11.) What individuals have been the most helpful to you during your predictive (genetic) testing process?**

- How have they been helpful?

**14.) How would you describe your thoughts and feelings about Huntington disease prior to receiving your predictive-test result?**

- How have your thoughts and feelings about Huntington disease changed, if at all, since receiving your predictive-test result?

**15.) How do you feel about your predictive (genetic) testing experience as a whole?**

**16.) After having this predictive (genetic) testing experience, what advice would you give someone who just received a predictive-test result similar to your own?**

**17.) Do you have any recommendations on how the predictive (genetic) testing process could be improved for others receiving a predictive test result like your own?**

*"This concludes our interview. Thank you very much for talking with me. Before I turn of the tape recorder, is there anything else you would like to tell me, or think I should know?"*

*"Do you have any questions?"*

*"Would you like the genetic counsellor working on this research team to call you to discuss any concerns or questions that you may have experienced during the interview?"*

*"In the future, if you have any questions or concerns, you may call a genetic counsellor at the Medical Genetics Clinic in your area. If needed, they will be able to direct you to additional resources in your area. The genetic counsellor in your area is (name) and can be reached at (number)."*

*"I would like to remind you that my contact information is on the consent form you have received for your records (sent with original mailed package). Should you have any questions about this interview or this research project, please do not hesitate to call me."*

*"Lastly, as mentioned previously, are you willing to be contacted in the future for a second interview. The purpose of this interview would be to clarify anything discussed today, ask you some additional questions, and/or share the results of this study with you for your opinion."*

*"I would like to thank you, once again for agreeing to participate in this study. Your contribution is very much appreciated and valued."*

## PARTICIPANT INTERVIEW GUIDE #2

**1. How have you been since you received predictive testing?**

**2. Can you tell me what it was like for you to be in a family that has Huntington Disease?**

- How old were you when you first found out about HD in your family?
- How does HD typically appear in your family (symptoms, age of onset (AOO))?
- What is your understanding of how HD is passed down in your family (inheritance)?
- Is there anything special or unusual about the HD in your family (symptoms, AOO, inheritance)?

**3. Is HD discussed in your family?**

- How is HD talked about in your family?
- When is HD talked about in your family?
- Who in your family talks about HD?
- How often is HD discussed in your family?
- What is discussed about HD (symptoms, AOO, inheritance, risks, predictive testing)?
- How old were you when HD was first talked about in your family?
- Can you share with me a recent conversation you have had about HD with a family member?

**4. How do you feel about having HD in your family?**

- How do you view the seriousness of HD in your family?
- How do your family members feel about having HD in the family?
- How do your family members view the seriousness of HD in the family?
- What is it like for you to watch your affected family members live with HD?
- (If applicable, what was it like for you to watch your family members die of HD?

***Ask the following questions if applicable to participant's family history (i.e. long-standing family history of HD with multiple affected family members)…***

- How do you think your experience would differ if the first person in your family to have HD were your sibling, instead of your parent and grandparent? Why do you think this would change your experience?

***Ask the following questions if applicable to participant's family history (i.e. new mutation family history, sporadic diagnosis of HD in sibling or parent)…***

- Did you know HD was in your family prior to your sibling's/parent's diagnosis?
- What was it like for you to have your sibling/parent diagnosed with HD when HD was not in your family before?
- How was your sibling/parent diagnosed with HD?
- What, if anything, were your sibling's (parent's) symptoms initially attributed to?
- What was your reaction to the HD diagnosis?
- How did you feel about the HD diagnosis?'
- Do you know how did your sibling/parent got HD if it was not in the family before?
- How do you think your experience would differ if you had a long-standing family history of HD with multiple affected family members? Why do you think this would change your experience?

**5. What is your understanding of the cause of HD?**

- What do you understand about the genetics of HD?
- What do you understand to be the genetic cause of HD?

- What is your understanding of how HD is passed down in families? Are there any other ways that HD can be passed down in families?
- Do you have to have a family history of HD to develop the disease? Why or why not?
- Where did you get your information about the (genetic) causes of HD?

**6. Can you tell me how you learned of your own risk to develop HD?**

- When did you find out you were at-risk to develop HD?
    - How old were you?
- How did you learn you were at-risk to develop HD?
    - From whom did you learn you were at-risk to develop HD?
- What was your reaction to learning that you were at-risk to develop HD?
- Prior to going for predictive testing, what did you believe was your risk to develop HD? Why?
- How did you feel about your risk to develop HD? Why?
- How serious of a risk did you feel it was?
    - Did you feel it was a small or a large risk? Why?

**7. How did you decide to go for predictive testing?**

- How did you learn that predictive testing was available?
- When did you decide to have predictive testing?
- Why did you decide to have predictive testing?
    - Was this an easy or difficult decision for you to make? Why?
- What did you think you would learn from predictive testing?
    - What information did you think you would learn about your own risk to develop HD?
    - What information did you think you would learn about your children's risk to develop HD?
- Prior to receiving your results, what did your genetic counsellor tell you about the possible predictive test results you could receive?
- Did you have a 'gut' feeling about the predictive test results you would receive?

**8. What were your results?**

- What did your results indicate about the possibility of you developing HD? Please explain further.
- What did your results indicate about the possibility of your children developing HD? Please explain further.
- Can you tell me how your result relates to your family history of HD?
- Who did you inherit your result from, your mom or your dad?

***Ask the following question if applicable to participant's understanding (i.e. understands a risk remains for their children to develop HD, even though they will not develop HD)…***

- Can you explain to me how it is possible for your children to develop HD but you will not

**9. How do you feel about your results?**

- How do you feel about the meaning of your result for your risk to develop HD?
- How do you feel about the meaning of your result for your children's risk to develop HD?
- How, if at all, has your result changed your feelings about HD?
- How would you have felt if you received a result that indicated you would develop HD? If your results indicated you would develop HD, what would that mean to you? How would that have impacted your life?
- How would you feel if you found out your children would develop HD? If you found out your children would develop HD, what would that mean to you?

***Ask the following question if applicable to participant's understanding (i.e. understands a risk remains for their children to develop HD, even though they will not develop HD)…***

- When you think of your children's risk to develop HD, what do you think of?
- How do you view the seriousness of the risk to your children? Do you consider their risk to be large or small? Why?
- What are some of the things you think about when determining the seriousness of the risk to your children?
- *Some people I have interviewed have talked about comparing their children's risk to develop HD to other health risks their children may have in order to determine the seriousness of the risk.* Have you done something like this? If so, what comparison have you made? Why?
- Do you think your family history has influenced how you view your children's risk to develop HD? How? Why?

**10. What was your reaction to your predictive test result?**

- What was the moment you heard your result like for you?
- How did you feel when you heard your results?
- What was your reaction to the meaning of your result for your risk to develop HD?
- What was your reaction to the meaning of your result for your children's risk to develop HD?
- Do you think you heard everything that was told to you about your result? Why or why not?
- *Some people I have talked to previously indicate that they "shut off" or stopped hearing what was being discussed once they heard their result.* Do you think you *"shut off"* once you heard your result? If so, why do you think you reacted this way?
    - How do you think "*shutting off"* once you heard your results influenced your ability to understanding of your results?
- *Some people I have talked to previously indicate that they felt shocked when they first heard their result.* Did you feel shock when you were first told your result? Why or why not?
    - In regards to your experience with HD and predictive testing, was there any other times you felt shocked? When? Why?
- How do you think your family history influenced the way you reacted to your result?
- How do you think the genetic counselling you received influenced the way you reacted to your result?

**11. Do you feel like you understand the meaning of your result? Why or Why not?**

- Do you feel any confusion about your result? What do you feel confused about?
- What aspect of your results would you like to know more about?
- How do you think your family history has influenced your understanding of your result?
- How do you think the genetic counselling you received influenced your understanding of your result?
- Has there been anything else that has influenced your understanding of your result?
- In what way, if any, do you think your understanding of your result could be improved?

**12. How do you feel about your predictive testing experience as a whole?**

- Do you feel predictive testing met your needs? Why or why not?
- Do you have any recommendations on how the predictive testing process could be improved for others receiving a predictive test result like the one you received?

**13. Is there anything else you would like to share with me or think I should know?**

**PARTICIPANT INTERVIEW GUIDE #3**

**Meaning about HD:**

1. Please tell me what words come to mind when you think of HD?

2. How would you complete the following sentence...
   - HD is ____ to me?
   - HD is ____ to my family?

3. What does HD mean to you?
   - What does HD mean to your family (your mother/father/siblings)?
   - How, if at all, is your family's view of HD different from what HD means to you?

4. How has your experience with HD affected your life?
   - Is there anything positive about your experience with HD?
   - How do you think your life would be different if you had never heard of HD?

**Family Experience:**

1. How old were you when you first found out about HD in your family?

2. What family experiences have you had with HD that you remember the most?
   - What stands out in your mind about your family experience with HD?

3. How is HD discussed in your family?
   - Who in your family talks about HD?
   - What is discussed about HD (symptoms, AOO, inheritance, predictive testing)?
   - How often is HD discussed in your family?
   - What was the first family discussion about HD you recall?
   - What other family discussions do you remember clearly?
   - What was the most recent family discussion about HD you had?

4. How does HD typically appear in your family (symptoms, AOO)?
   - Is there anything special or unusual about the HD in your family (symptoms, AOO, inheritance)?

***Ask the following questions if applicable to participant's family history (i.e. new mutation family history, sporadic diagnosis of HD in sibling or parent)…***

- Did you know HD was in your family prior to your sibling's/parent's diagnosis?
- What was it like for you to have your sibling/parent diagnosed with HD when HD was not in your family before?
- How was your sibling/parent diagnosed with HD?
- What, if anything, were your sibling's (parent's) symptoms initially attributed to?
- What was your reaction to the HD diagnosis? Were you shocked at the diagnosis? Why or why not?
- How did you feel about the HD diagnosis?
- Do you know how did your sibling/parent got HD if it was not in the family before?
- How do you think your experience would differ if you had a long-standing family history of HD with multiple affected family members? Why do you think this would change your experience?
- How do you think your experience would differ if you had a strong family history of HD with multiple affected family members? How do you think this would change your experience? Why?
- *Some individuals with a similar family history to yours have described HD in their family as being "out of the blue".* Thinking about your family experience with HD, does that phrase mean anything to

you? What does it mean to you? If not, what word or phrase would you use to describe HD in your family?

***Ask the following questions if applicable to participant's family history (i.e. long-standing family history of HD with multiple affected family members)…***

- How do you think your experience would differ if you had a parent/sibling diagnosed with HD but no other affected family members? How do you think this would change your experience?
- *Some individuals with a similar family history to yours have described it as "growing up with HD".* Thinking about your family experience with HD, does that phrase mean anything to you? What does it mean to you? If not, what word or phrase would you use to describe HD in your family?

**Learning Process:**

1. How did you first learn about HD?
    - From whom did you first learn about HD?
    - How old were you?

2. How did you first learn you were at-risk to develop HD?
    - From whom did you first learn of your risk?
    - How old were you?
    - How long after learning about HD did you learn of your risk?

3. How did you first learn about the availability of predictive testing?
    - From whom did you first learn of predictive testing?
    - How old were you?
    - How long after learning about your risk did you learn about predictive testing?

**Understanding of HD:**

1. What is your understanding of the cause of HD?

2. What do you understand about the genetics of HD?
    - What is the genetic cause of HD?
    - How is the genetics of HD related to your previous risk to develop HD?

**3. What is your understanding of how HD is typically passed down in a family?**
    - What word or phrase would you say describes how HD is passed down in a family?
    - How is HD passed down in your family?
    - Is there any other way that HD can be passed down in families?
    - Do you have to have a family history of HD to develop the disease? Why or why not?

***Ask the following questions if applicable to participant's family history (i.e. new mutation family history, sporadic diagnosis of HD in sibling or parent)…***

- How did your sibling/parent got HD if it was not in the family before?
- How is the genetics of HD related to your parent or siblings diagnosis?

**Predictive Testing:**

1. Prior to going for predictive testing, what did you believe your risk to develop HD was?
    - How did you feel about your risk to develop HD?
    - How serious of a risk did you feel it was?
        - Did you feel it was a small or a large risk? Why? Compared to what?

2. Prior to receiving your results, what results did you think you could receive through predictive testing?
- Did you have a 'gut' feeling about what result you would receive?

**Receiving Predictive Test Result:**

1. What predictive test result did you receive?
   - What does your result indicate about the possibility of you developing HD?
   - What did your result indicate about the possibility of your children developing HD?
   - Did your predictive test result have a special name?
   - What word or phrase would you use to describe your predictive test result?

2. What was the moment you received your predictive test result like for you?
   - What was your reaction to receiving this result?
   - Where you shocked at your result? Why?
     - Have you felt shock at any other time during your experience with HD and predictive testing?
   - What questions did you have about your result?

3. *Some people I have talked to say that they "shut off" or stopped hearing their genetic counselling after they heard their predictive test result.* Do you think you *"shut off"* once you heard your result? Why?

4. How did you feel about your predictive test result?
   - Why did you feel that way?
   - What do you think influenced your feelings about your result; your family, your beliefs, the genetic counselling you received?

**Understanding of Predictive Test Result:**

1. Do you feel you understanding your predictive test result?
   - What do you not understand about your result?
   - What would you like to understand better?
   - How do you think your understanding could be improved?

2. How did your result fit with your previous understanding of HD and its genetics?
   - What do you think has influenced your understanding of your results; your family, your previous beliefs, the genetic counselling you received?
   - If you experienced *"shutting off"*, how do you think this influenced your understanding of your results?

3. Do you understand how your predictive test result relates to your family history of HD?
   - Who did you inherit your result from, your affected or non-affected parent?
   - How does your result relate to your children's risk?

**Meaning about Predictive Test Result:**

1. Please tell me what words come to mind when you think of your predictive test result?

2. How would you complete the following sentence...
   - My predictive test result is ____ to me?
   - My predictive test result is ____ to my family?

3. What does your predictive test result mean to you?
   - What does your predictive test result mean to your family (your mother/father/siblings)?
   - How, if at all, is your family's view of your result different from what your result means to you?

4. How has your predictive test result change the meaning of HD for you?
    - How has the meaning of HD changed from when you first became aware of HD?
    - What may change the meaning of HD for you in the future?

5. How has your predictive test result affected your life?
    - How do you think your life would be different if you received a result that indicated you would develop HD?

***Ask the following questions if applicable to participant's understanding (i.e. understands a risk remains for their children to develop HD, even though they will not develop HD)…***

- How do you think your life would be different if your received a result that indicated your children were not at-risk to develop HD

**Recommendations:**

1. Do you feel predictive testing met your needs? Why or why not?
    - Do you have any recommendations on how the predictive testing process could be improved for others receiving a predictive test result like the one you received?

**Conclusion:**

1. Is there anything else you would like to share with me or think I should know?

**PARTICIPANT INTERVIEW GUIDE #4**

***'Grey':***

***Ask the following questions if applicable to participant's previous interview and understanding…***

*- Many individuals in this study describe their predictive test result as a "grey" result.* Do you think the term *"grey"* accurately captures what your result means to you? Why or why not?
- If yes, what does the term *"grey"* mean to you? If no, what would be a better word or phrase? Why?
- *Some people in this study have indicated that they use the term "grey" to describe the uncertainty their result poses for the future health of their children (i.e. will my children get HD or won't they?).* Do you agree with this? Why or why not?
- *Other people have indicated that they use the term "grey" to describe the uncertainty of their result due to our limited scientific knowledge.* Do you agree with this? Why or why not?
- *Additional people have indicated that they use the term "grey" to describe the general uncertainty they feel about the meaning of their result*. Do you agree with this? Why or why not?

***Ask the following questions if applicable to participant's previous interview and understanding…***

*- During our previous interview, you described your predictive test result as a "grey" result.* What does the term *"grey"* mean to you?
- Where did you learn this term?
- Did you or someone you know call your result *"grey"*?
- Did your genetic counselling call your result *"grey"*?
- Did you read about *"grey"* results somewhere? Where did your read about *"grey"* results?

***'Grasping the Grey':***

1.) *Some people in this study have indicated that they struggled to understand the meaning of their result*. Did you struggle to understand your result?

- If yes, what have you struggled to understand about your result?
- Why do you think you have struggled?
- How has this struggle made you feel?
- What do you think could have been done in your genetic counselling so that you would not have struggled as much to understand your result?
- Do you think you are still struggling to understand your result? If so, what are you still struggling with?
- What could help you overcome this struggle?
- If no, why do you think you did not struggle to understand your result?

- What do you think was done in your genetic counselling that helped you to understand?
- Did you have some previous knowledge that helped you to understand? What was that knowledge?
- Did you find some resources that helped you to understand? What were those resources?

2.) *I have labeled the struggle that some individuals underwent to understand their result as "grasping the grey".* Do you think this is an accurate phrase to use? Why or why not?

- If yes, what does the term *"grasping"* mean to you?
- If no, what would be a better word or phrase? Why?

***Family Experience:***

1.) Do you think your family experience influenced your understanding/interpretation of your result? Why or why not?

2.) How do you think your family experience contribute to you struggle to understand/interpret your result?

3.) *Some individuals in this study have described their family experience with HD as "out of the blue" meaning that HD occurred in their family unexpectedly, in fact many had never heard of HD before.* How do you think this familial experience would influence an individual's ability to understand their *"grey"* result?

- *Other individuals in this study have indicated that they "grew up" with HD, meaning that they have always known HD was in their family and that they were at-risk of the disease.* How do you think this familial experience would influence an individual's ability to understand their *"grey"* result?
- Thinking about these two different family experiences, which family experience do you think would increased an individual's struggle to understand their *"grey"* result? Why?
- Which family experience do you think would decrease an individual's struggle to understand their *"grey"* result? Why?

***Beliefs & Expectations:***

1.) When thinking about HD predictive test results, what does the phrase *"black or white"* mean to you?

- When thinking about HD inheritance, what does the phrase *"50:50"* mean to you?

2.) *Some individuals shared other beliefs about HD that existed in their family.* Did your family have other beliefs about HD and how it was passed down in the family? If so, what were those beliefs?

3.) What results were you expecting from your genetic testing?

- Did you expect *"grey"* result? Why or why not?
- How do you think your expectation of genetic testing influenced your understanding of your result?

4.) *Many people have indicated that their genetic counselling focused on whether they inherited the HD gene or not.* Does this reflect your experience?

- Do you think this focus is appropriate? Why or why not?

5.) What was your reaction to hearing your result?

- Why did you react this way?

6.) How did you feel about your result?

- Could you tell me a little more about why you felt this way?

7.) Many people have said their result was unexpected, how was it for you?

- *These people say that because their result was unexpected they experienced shock.* Did you experience shock? What was this like for you?
- Do you feel like you heard everything about your result? Why or why not? What do you think you might have missed?
- Do you think feeling shocked contributed to your struggle to understand your result? If yes, in what way?
- Were there other emotions you experienced when you received your result that influenced your understanding of your results? If so, what were those emotions/feelings?

***Meaning/Interpretation:***

1.) *Individuals in this study interpreted the meaning of their grey result in four different ways; to some people a grey result meant they and their family were "free & clear" of HD; other people where "uncertain" about the meaning of a grey result and were still struggling to understand it; some other people thought their result "could have been worse"; and lastly, some individuals thought their grey result was a "threat" to their children's and/or family's future.*

- When thinking about these four different meanings of a grey result, where do you see yourself fitting?
- Why do you think you fit there?
- Has the meaning of your result changed since we last spoke? If so, what has changed? What has caused this change?

***Ask the following questions based on the meaning/interpretation participant's indicate above…***

***A. Uncertainty:***

- What about your result are you uncertain about?
- Why do you think you feel uncertainty about your result?
- How does your uncertainty make you feel?
- What would help address your uncertainty?
- What information about your result are you uncertain about?
- Why do you think you are uncertain about this information?
- Do you think your family experience has contributed to the uncertainty you feel? If so, how?
- Do you think the genetic counselling you received contributed to the uncertainty you feel? If so, how?
- What else, if anything, has contributed to your uncertainty?
- *If you recall, I called the struggle individuals undergo to understand their "grey" result "grasping the grey".* Do you think this phrase could also be used to describe your struggle with the uncertainty you feel about your result?

***B. It Could Be Worse:***

- How do you think your result could be worse?
- For whom could your result be worse (i.e. yourself, your children, your family)?
- In what way could your result be worse?
- In your opinion, what would be the worse-case scenario?
- What would be the best-case scenario?
- When you interpreted your result as something that could be worse, what did you compare it to?
- How do you feel about your result?
- Despite feeling that your result could be worse, do you still worry about what your result means? What do you worry about?
- *Some individuals have discussed keeping their worry "in the back of their mind".* Does that phrase describe you? Why or why not? If yes, what does that phrase mean to you?
- Do you feel any uncertainty about your result? If so, what are you uncertain about? Why do you think you are uncertain?
- Do you think your family experience has influenced your view that your result could be worse? If so, how?
- *For some people in this study they, interpreted their result to mean they and their children/family are 'free and clear' of HD.* Do you feel that way? Why do you think these individuals may feel this way about their result?

- *Other individuals in this study felt their result meant that their children and/or family had a "threatened future". These people were very worried about their result*. Do you feel that way? Why do you think these individuals may feel this way about their result?

***C. Threatened Future:***

- How is your result a threat?
- For whom is your result a threat?
- Why is your result a threat?
- Would you view a gene-positive result also as a threat?
- How did your family experience impact the meaning of your result as a threat?
- How does being a man/woman influence the meaning of your result as a threat?
- How do you feel about your result?
- Do you worry about your result on a daily basis?
- *Some individuals have discussed living with the worry about the risk to their children "in the forefront of their mind".* Does that phrase describe your worry? What does that phrase mean to you?
- Do you feel any uncertainty about your result? If so, what are you uncertain about? Why do you think you are uncertain?
- *Some people in this study interpreted their result to mean their children/family are "free and clear" of HD.* Why do you think they felt this way?
- *Other individuals in this study felt their result "could have been worse"*. Why do you think they felt this way?
- *Other people in this study were uncertain about the meaning of their result*. Why do you think they feel uncertain?

***Conclusion:***

- If there was one piece of advice you could share with genetic counsellors about *"grey"* results, what would that be?
- Is there anything else you would like to share with me or think I should know?

THE UNIVERSITY OF BRITISH COLUMBIA

**MEDICAL SERVICE PROVIDER LETTER OF INVITATION**

**DEVELOPMENT OF HUNTINGTON DISEASE PREDICTIVE TESTING GUIDELINES**

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**[Date]**

Dear **[Participant Name]**

We are writing to invite you to participate in an important study on predictive (genetic) testing for Huntington disease. The purpose of this study is to learn more about the experience of individuals who have received an intermediate allele predictive-test result for Huntington disease. Currently, there is no information in the literature about the psychological and social experience of individuals who have received an intermediate allele result. Clinical experience has suggested that patients who receive this predictive-test result often struggle to understand its clinical implications and have difficulties explaining this result to other family members. Through this research, we hope to learn how to better support, educate, and counsel individuals who receive an intermediate allele test result.

Your name was obtained from the Canadian Association of Genetic Counsellors membership list. You have been invited to participate in this study as the membership list indicated that your practice area involves adult genetics. Specifically, we are inviting genetic counsellors who routinely provide genetic counselling to individuals undergoing predictive (genetic) testing for Huntington disease. If you do not routinely provide genetic counselling for individuals undergoing predictive (genetic) testing for Huntington disease, please decline from participating in this study by checking the appropriate box on the accompanying consent form, which indicates that you do not practice in this area. Please return the consent form using the enclosed self-addressed stamped reply envelope.

This study is being led by principal investigator, Dr. Michael Hayden, at the University of British Columbia and his graduate student, Ms. Alicia Semaka. Participation in this study involves an interview, which will take approximately one hour to complete. The interview will ideally take place in-person, at your place of work. If an in-person interview is not convenient, the interview can be conducted over the telephone. The interview will consist of questions regarding your experience with providing genetic counselling to individuals found to have an intermediate allele.

There is no obligation to take part in this research study. If you do not wish to participate, please discard this information. Your employment will not be affected in anyway if you decline. Additionally, at any time after consenting to participate, you may withdraw from the study.

If you would like to be involved in this study, all information obtained will be kept strictly confidential. Your anonymity will be protected at all times by using a code number as an identifier and keeping all information in a secure location available only to members of the research team.

If you are willing to participate in this study, please sign and return the enclosed consent form using the self-addressed, stamped envelope. Upon receiving your consent form, you will be contacted to arrange a time, date, and location, which will be convenient for you to privately participate in the interview.

If you have any questions regarding the research, please feel free to contact Ms. Alicia Semaka at **(XXX) XXX XXXX**. Thank you for your time and consideration.

Sincerely,

Michael R. Hayden MB, ChB, PhD, FRCP(C), FRSC
University Killam Professor,
University of British Columbia, Department of Medical Genetics
Director and Senior Scientist,
Centre for Molecular Medicine and Therapeutics

Alicia Semaka MSc, CGCC, CGC
Medical Genetics Doctoral Candidate
Genetic Counsellor
University of British Columbia
Centre for Molecular Medicine and Therapeutics

THE UNIVERSITY OF BRITISH COLUMBIA

**MEDICAL SERVICE PROVIDER CONSENT FORM**

**DEVELOPMENT OF HUNTINGTON DISEASE PREDICTIVE TESTING GUIDELINES**

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**Principal Investigator:**

Dr. Michael Hayden
University Killam Professor
University of British Columbia
Department of Medical Genetics
Centre for Molecular Medicine and Therapeutics
**(XXX) XXX XXXX**

**Co-Investigators:**

Alicia Semaka, MSc
Medical Genetics Doctoral Student
University of British Columbia
Department of Medical Genetics
Centre for Molecular Medicine and Therapeutics
**(XXX) XXX XXXX**

Dr. Lynda Balneaves
Assistant Professor
University of British Columbia
School of Nursing
**(XXX) XXX XXXX**

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## WHAT IS THE PURPOSE OF THIS STUDY?

We are inviting you to participate in an important study on individuals who have undergone predictive (genetic) testing for Huntington disease (HD). The purpose of this study is to learn more about the experience of individuals who have received intermediate allele predictive-test results. This research is being performed as a requirement of a postgraduate degree in Medical Genetics and the results of this study will reported in the student's dissertation.

Currently, there is no information in the literature about the psychological and social experience of individuals who have received an intermediate allele predictive-test result. Clinical experience has suggested that patients receiving this predictive-test result often struggle to understand its clinical implications and have difficulties explaining this result to other family members. Through this research, we hope to learn how to better support, educate, and counsel individuals who receive an intermediate allele result. Additionally, this study will help to guide the development of new Huntington disease predictive (genetic) testing guidelines, which specifically address the needs of individuals found to have an intermediate allele result and their medical genetics professionals.

## WHAT DOES THIS STUDY INVOLVE?

Participation in this study involves an interview, which will take approximately one hour to complete. The interview will ideally take place in-person at your place of work. If an in-person interview is not convenient, the interview can be conducted over the telephone.

The interview will consist of questions regarding your experience with providing predictive (genetic) testing for individuals found to have an intermediate allele. The interview questions will be open-ended, in order to allow you to speak freely, and share as much, or as little as you feel comfortable in doing.

With your permission, we would like to audiotape the interview in order to transcribe the conversation for analysis. If you are not comfortable with this, the interview will not be recorded. Furthermore, if you would like the tape recording to be stopped at any time during the interview, this will be arranged.

You may be contacted to participate in a follow-up interview. The purpose of a second interview would be to clarify anything discussed in the first interview, ask you some additional questions, and/or share the results of this study with you for your opinion. A follow-up interview will take approximately ½ hour and will be tape-recorded. This interview may be conducted in-person or over the telephone. Involvement in a follow-up interview is not required for participation in this study.

**HOW DO YOU BECOME INVOLVED IN THIS STUDY?**

If you wish to participate in this study, please sign and return this consent form using the enclosed stamped, self-addressed envelope by **[DATE]**. Once we have received your consent form, you will be contacted to arrange a time, date, and location that will be convenient for you to participate in the interview.

There is no obligation to take part in this research study. If you do not wish to participate, please discard this information. Your employment will not be affected in anyway if you decline. Additionally, at any time after consenting to participate, you are free to withdraw from the study.

If you are refraining from participating in this study because you do not routinely provide genetic counselling for individuals undergoing predictive (genetic) testing for Huntington disease, please indicate this on the consent form and return it using the enclosed stamped, self-addressed envelope.

**WHAT ARE THE RISKS AND BENEFITS OF THIS STUDY?**

You will not receive any direct benefit from taking part in this study. However, we think the results of this study will help improve predictive (genetic) testing for Huntington disease for individuals who receive an intermediate allele result and provide guidance for medical genetics professionals on how to best support, educate and counselling these individuals. There are no expected risks related to participation in this study and it is unlikely that you will experience any psychological distress from participating in the interview.

**WHAT ABOUT CONFIDENTIALITY?**

Your participation in this study will be kept confidential to the extent permitted by law. Your anonymity will be protected at all times by using a code number as an identifier and keeping all information in a locked file cabinet, available only to members of the research team. All computer files will be password protected. When transcribing the audiotaped interview, all names and any identifying information will be removed. The interview transcripts and audiotapes will be kept for the duration of 5 years, in compliance with University of British Columbia research policy. After this time, they will be destroyed in a manner that will ensure confidentiality. In the event of any report or publication from this research, the identity of the participants will not be revealed and the results will be summarized in a manner that participants cannot be identified.

**WHAT IF YOU HAVE QUESTIONS?**

We welcome any questions you may have about this study. If you have any questions or you wish to withdraw your initial consent, please feel free to contact the study coordinator, Ms. Alicia Semaka at **(XXX) XXX XXXX**.

If you have any questions or concerns about your treatment or rights as a research subject, please contact the Research Subject Information Line in the University of British Columbia's Office of Research Services, at **(XXX) XXX XXXX**.

**********

Your signature below indicated that you have read the above information, understand the risks, benefits, and procedures of the study, and voluntarily agree to participate in this research project.

Please keep one copy of this consent form for your records and return the other copy of the consent form using the self-addressed, stamped envelope included by **[DATE]**.

| | |
|---|---|
| Participant Name (Please Print) | Telephone Number |
| Signature | Date |

THE UNIVERSITY OF BRITISH COLUMBIA

**MEDICAL SERVICE PROVIDER INTERVIEW GUIDE**

**DEVELOPMENT OF HUNTINGTON DISEASE PREDICTIVE TESTING GUIDELINES**

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**Time of Interview:**

**Date:**

**Location:**

**Interviewee:**

**Interview Code:**

*"Thank you for agreeing to participate in this interview. My name is Alicia Semaka. I am a medical genetics doctoral student. This research is being performed as part of my dissertation. Once again, the purpose of this study is to learn more about the predictive (genetic) testing experience of individuals who have received intermediate allele results for Huntington disease."*

*"If for any reason you no longer wish to participate in this study, you are under no obligation and can do so with out consequence. Would you like to proceed with the interview?"*

*"Please remember that if you would like to stop the interview at anytime or would like to take a break, you are free to do so. We can always schedule an alternate time to complete the interview, if you wish."*

*"I would like to remind you I would like to audio tape this interview in order to transcribe our conversation for easier analysis. If you are not comfortable with this, please let me know at this point and our conversation will not be recorded. Furthermore, if you would like me to turn off the recorder at any point during the interview, just let me know."*

*"Lastly, please keep in mind that everything you say during this interview will be kept strictly confidential and will only be shared with members of the research team. Your name and all identifying information will be removed. Your interview transcript will receive a code number that will not identify you in anyway. After the interview is transcribed, all audiotapes will be destroyed. All sensitive material obtained during this research study will be stored in a secure location."*

*"This interview will take approximately one hour to complete. I will be using an interview guide to help ensure all topic areas are discussed in each interview. The questions in this interview are open-ended, so please speak freely, and share as much, or as little as you feel comfortable in doing. Furthermore, if you have any questions during the interview, please ask."*

*"With your permission, I may ask you speak to you again at a later date. The purpose of a second interview would be to clarify anything discussed today, ask you some additional questions, and/or share the results of this study with you for your opinion."*

*"Before we begin, do you have any questions?"*

## DEMOGRAPHIC INFORMATION

1.) Gender:

☐ Male ☐ Female

2.) What is your present position (i.e. head genetic counsellor, head of genetic counselling program, etc.)?

3.) When did you begin working as a genetic counsellor? _ _ / _ _ / _ _ _ _
(MM / DD / YEAR)

4.) From which school did you receive your genetic counselling training? _____

5a.) Do you have any additional or previous training (i.e. RN)?

☐ Yes ☐ No

5b.) If yes, please specify? _____

6a.) Are you board certified or eligible for certification?

☐ Yes ☐ No

6b.) Which certifications do you hold?

☐ CCGC (Canadian) ☐ CGC (American)

7.) How long have you been providing genetic counselling for individuals undergoing predictive testing for Huntington disease?

___ Years

8a.) In additional to providing genetic counselling for individuals undergoing predictive (genetic) testing for Huntington disease, do you practice in other specialty areas (i.e. prenatal or cancer counselling)?

☐ Yes ☐ No

8b.) If yes, please specify area of practice? Indicate what proportion of time you spent in each practice area?

___ General Genetics ___ Prenatal ___ Pediatric

___ Biochemical ___ Cancer ___ Research

___ Other: Please Specify _____

8c.) Approximately how many patients do you provide genetic counselling for predictive (genetic) testing for HD?

___ per month

___ per year

9.) Is the Medical Genetics Clinic in which you currently work, associated with a teaching hospital and/or a University?

☐ Yes ☐ No

10.) Does your Medical Genetics Clinic have an affiliated Genetic Counselling Program?

☐ Yes ☐ No

11.) If yes, do you provide supervision to genetic counselling students when providing genetic counselling for individuals undergoing predictive (genetic) testing for Huntington disease?

☐ Yes ☐ No

12a.) Is the Medical Genetics Clinic in which you work, a specialty clinic for Huntington disease (i.e. provide routine medical care for individuals affected with HD)?

☐ Yes ☐ No

12b.) If yes, what other services does this clinic provide?

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**MEDICAL GENETICS SERVICE PROVIDER INTERVIEW GUIDE #1**

**1.) Please tell me about your experience with providing genetic counselling for individuals undergoing predictive (genetic) testing for Huntington disease.**

**2.) Please tell me about your experience with providing genetic counselling for individuals found to have an intermediate allele for Huntington disease.**

**3.) In your own words, how would you define an intermediate allele?**

**4.) Tell me how you go about counselling an individual found to have an intermediate allele predictive-test result?**

- What information do you provide to the patient?
- When do you commonly provide this information (i.e. which genetic counselling session)?
- How do you communicate this information regarding intermediate alleles for Huntington disease (i.e. do you use diagrams, patient pamphlets, etc)?

**5.) What is <u>your</u> understanding of the clinical implications an intermediate allele predictive-test result for your patient and their children and extended family members?**

- How well do <u>you</u> feel you understand the clinical implications an intermediate allele predictive-test result for your patient and their children and extended family members?
- How could <u>your</u> personal understanding of intermediate allele predictive-test result could be improved, if at all?

**6.) In your opinion, how do you think <u>patients</u> understand the clinical implications of an intermediate allele for themselves, their children, and their extended family members?**

- In your experience, what, if anything, do <u>patients</u> struggle to understand about intermediate alleles for Huntington disease?
- What, if anything, do <u>patients</u> easily understand about intermediate alleles for Huntington disease?
- Do certain people understand their intermediate allele predictive-test result better than others? Who?
- How do you think <u>patient</u> understanding of intermediate allele predictive-test result could be improved, if at all?

7.**) In your experience, what are the psychosocial issues faced by intermediate allele carriers?**

- How are these psychosocial issues different to those faced by individuals receiving a positive or negative predictive-test result?
- How are these psychosocial issues similar to those faced by individuals receiving a positive or negative predictive-test result?

**8.) In your experience, what has been the reaction of your patients to an intermediate allele result?**

- Do certain people respond different to an intermediate allele result? Who?
- How do they respond?

**9.) How do you think this predictive-test result has influenced the lives of your patients (i.e. how has it influenced their decision making for reproductive or employment decisions, if at all)?**

**10.) What are some of the common questions you are asked when communicating an intermediate allele predictive-test result?**

**11.) What resources, if any, do you use when preparing to communicate intermediate allele predictive-test results?**

**12.) What difficulties [problems/concerns/challenges], if any, have you encountered when providing genetic counselling to individuals found to have an intermediate allele?**

**13.) When you look back at all the cases involving intermediate allele results, is there anything that stands out in your mind?**

**14.) Reflecting on your experience counselling intermediate allele carriers, what advice would you give a new graduate or someone new to providing genetic counselling for Huntington disease predictive (genetic) testing?**

**15.) Is there anything else about providing predictive-test results to intermediate allele carrier that has occurred to you that you would like to share?**

*"This concludes our interview. This concludes our interview. Do you have any questions?"*

*"I would like to remind you that my contact information is on the consent form you have received for your records (sent with original mailed package). Should you have any questions about this interview or this research project, please do not hesitate to call me."*

*"Lastly, as mentioned previously, are you willing to be contacted in the future for a second interview. The purpose of this interview would be to clarify anything discussed today, ask you some additional questions, and/or share the results of this study with you for your opinion. I would like to thank you,*

*once again for agreeing to participate in this study. Your contribution is very much appreciated and valued.*

## MEDICAL GENETICS SERVICE PROVIDER INTERVIEW GUIDE #2

**1. Please tell me about your experience with providing genetic counselling for individuals undergoing predictive testing for Huntington disease.**

**2. Please tell me about your experience with providing genetic counselling for individuals found to have an intermediate allele.**

- How does the genetic counselling you provide to individuals with either a positive or negative result differ from the counseling you provide to those individuals with an intermediate allele result?
- Are there any recent cases that had an intermediate allele that stand out in your mind? If so, can you please tell me about these cases?

**3. What are your counselling practices regarding intermediate alleles?**

- What information on intermediate alleles do you provide your patients?
- What CAG sizes does your centre consider to be an intermediate allele?
- What quantified risk does your centre quote patients for the likelihood of intermediate allele expansion into the HD range? Do you quote different risks based on the gender of the patient?
- How do you normally communicate information on intermediate alleles to patients (i.e. diagrams, verbal, patient pamphlets)?
- What resources do you use for information on intermediate alleles?
- When do you commonly provide this information (i.e. pre or post results)?
- Do you provide this information to all patients undergoing predictive testing? If not, how do you determine whether or not you will share information on intermediate alleles with the patient?

**4.** ***Some other counsellors I have spoken to have indicated that they only provide information on intermediate alleles when the patient has a new mutation family history but do not provide information on intermediate alleles when a patient has a traditional long-standing family history). The rationale they gave for providing different information on intermediate alleles based on family history is that in a new mutation family history, intermediate alleles must be discussed in order to explain the sporadic case of HD in the family, whereas in a traditional long-standing family history, the counselling must focus on preparing the patient to receive either a positive or negative result.***

- Do these counselling practices reflect the counselling that your centre provides?
- Do you agree with this rationale for providing different information on intermediate alleles based on family history? Why or why not?

**5. In your experience, do you think patients understand the clinical implications of an intermediate allele? Why or why not?**

- What information do patients struggle to understand about intermediate alleles for Huntington disease?
- In your opinion, do you think certain people understand their intermediate allele predictive test result better than others? Who? Why?
- What do you think are some of the factors that influence whether or not a patient understands the intermediate allele result?
- How do you think patient understanding of intermediate alleles could be improved, if at all?

**6.** ***In the interviews I have conducted, patient understanding about intermediate alleles and their clinical implications is variable. In particular, some patients are unaware of the implication for their children despite receiving counseling about this implication.***

- Why do you think this may be the case?

**7.** ***One of factor that appears to influence patient understanding of intermediate alleles is the patient's family history. Specifically, those individuals that have a traditional long-standing family history do not understand the implications of an intermediate allele for their children compared to those individuals that had a new mutation family history.***

- Why do you think family history may influence a patient's understanding of their intermediate allele result and its implications?

**8.** ***The data collected so far suggests that individuals with a traditional long-standing family history may not be aware of the implication for their children because they thought about their children's risk within the context of the traditional autosomal dominant inheritance pattern of HD and reasoned that their children's risk was eliminated because they would not develop HD.***

- Does this seem like a likely explanation for the observed difference in patient understanding about intermediate alleles based on family history? Why or why not?

**9. In your experience, what has been the reaction of your patients when they receive an intermediate allele result?**

- What has been the reaction of your patients to the implications of an intermediate allele for their own risk to develop HD?
- What has been the reaction of your patients to the implications of an intermediate allele for their children's risk to develop HD?
- Do you think certain patients respond differently to an intermediate allele result? Who? Why do you think these patients respond the way they do?
- What are some of the common questions you are asked when communicating an intermediate allele predictive test result?

**10.** ***Some people I have spoken to have talked about "shutting down" and not being able to hear what is being said immediately after finding out they will not develop HD*****.**

- In your experience, is this a common reaction for patients undergoing predictive testing?
- If so, why do you think people *"shut down"*?
- How do you think *"shutting down"* influences individuals' understanding of the implication of an intermediate allele for their children?
- How do you think this reaction of shutting down could be prevented or minimized, if at all?

**11.** ***Some people I have spoken to indicate that they felt shock when they were told they receive an intermediate allele result.***

- In your experience, are feelings of shock a common reaction for individuals who receive an intermediate allele? Why or why not?
- How do you think feelings of shock impact the patient's ability to understand the implication of an intermediate allele result?

**12.** ***The participants who expressed feelings of shock at hearing their result were a small subset of individuals who had a traditional long-standing family history that were aware of the implication of an intermediate allele for their children. These individuals indicated that they experienced shock because they thought that HD inheritance was 50:50. The following quote demonstrates this belief… "It has always been my understanding that if I did not develop it, than to that extent the children would be free of it too."***

- Do you think the widespread belief that HD inheritance is 50:50 influences patient understanding of intermediate alleles? Why or why not?
- Do you think efforts should be made to educate the HD community that HD inheritance is not always 50:50? Why or why not?

**13. In your opinion, what are some of the psychosocial issues faced by individuals with an intermediate allele?**

- How are these psychosocial issues similar to those experienced by individuals receiving a positive or negative predictive-test result? How are they different?
- How can we prepare/support individuals with an intermediate allele with these psychosocial issues?

**14. How do you think this predictive-test result has influenced the lives of your patients in terms of their reproductive decision-making?**

**15.** ***Some intermediate allele carriers have expressed the desire to have prenatal diagnosis to determine if the intermediate allele expanded into the HD range.***

- What is your opinion of this request?
- What ethical dilemmas do you foresee in this regard?

**15. What are some of the difficulties or challenges you have encountered when providing genetic counselling to individuals found to have an intermediate allele?**

***16. Given our current understanding of intermediate alleles, there is an element of uncertainty about the likelihood of expansion into the HD range.***

- How does this uncertainty pose a challenge to you when counselling individuals found to have an intermediate allele?
- How can we provide effective counselling to patients in the face of this uncertainty?

**17.** ***The intermediate allele CAG size range has varied over the last decade, thus, it is possible that some individuals who were told that they have a 'negative' predictive test result now in fact have an intermediate allele.***

- In your opinion, do we have a duty to recontact these individuals and provide them counselling based on the reinterpretation of their CAG size? Why or why not?
- Do you think this is in the best interest of the patient? Why or why not?

**18.** ***In the field of medical genetics, there can be an influx of new understanding gained from bench research that sometimes conflicts with what was previously believed.***

- In your opinion, what is the most effective way to get this new information from 'bench to bedside'?
- What are some of the challenges you would foresee in getting the new information into clinical practice?
- How could we ensure that the new understanding is adopted by all medical genetics clinics so that there are consistent counselling practices?

**19.** ***Some counsellors have mentioned the idea of a reduced predictive testing protocol for children and/or extended family members, particularly if the intermediate allele is identified in the non-HD branch of the family since the likelihood of identify an HD gene is decreased.***

- What is your opinion of this?

**20. Is there anything else about providing predictive-test results to a person with an intermediate allele test result that you think would be important for me to know?**