MOLECULAR GENETICS REPORT:
Huntington Disease Testing via the HTT Repeat Expansion Test

SUMMARY OF RESULTS: Positive for 39 CAG Repeats
One Reduced Penetrance Allele

RESULTS AND INTERPRETATIONS: This patient is heterozygous for one HTT normal allele with 15 CAG repeats and one expanded HTT allele with 39 CAG repeats. This finding is consistent with a reduced penetrance Huntington Disease allele (e.g., Jama et al. 2013. J. Mol. Diag. 15:255-262; Bean & Bayrak-Toydemir, 2014. Genet. Med. 16:e2. doi: 10.1038/gim.2014.146). Although we are unable to draw any conclusions regarding the potential development of Huntington Disease (HD) in this patient, some data is available to assist in determining the probability of onset of HD based on age and CAG repeat size (Brinkman et al. 1997. Am. J. Hum. Genet. 60:1202-1210; http://www.cmmt.ubc.ca/sites/default/files/pdf_hayden_supplementary_tables.pdf).

These results should be interpreted in the context of clinical findings, family history and other laboratory data. All genetic tests have limitations. Please see limitations and other notes for this particular test on pages 2-4.

NOTES: Genetic counseling is recommended. Testing for the HTT CAG repeat expansion is available for at-risk, adult family members. Our testing method is not validated for prenatal specimen types.

METHODS: Using this patient’s genomic DNA, we tested for the HTT repeat expansion using a combination of two complementary analyses: (1) a repeat-primed PCR assay, as previously described (Warner et al. 1996. J. Med. Genet. 33:1022-1026; Kalman et al. 2007 Genet. Med. 9:719-723; Jama et al. 2013. J. Mol. Diag. 15:255-262) and (2) a fluorescent fragment-length PCR assay using HTT specific primers, as previously described (Jama et al. 2013. J. Mol. Diag. 15:255-262). Test controls included DNA samples from patients known to have HTT repeat expansions as well as from healthy controls. Transcript Number: HTT (NM_002111.7)
**SUPPLEMENTAL INFORMATION**

**V.17.03**

**SCREENING FOR THE HTT CAG TRINUCLEOTIDE REPEAT EXPANSION**

**Limitations of Test**

This test is designed to only detect pathogenic expansions of a CAG trinucleotide repeat in exon 1 of the *HTT* gene. At this time, no other causative variants within this gene have been conclusively documented to be pathogenic; therefore testing this gene for other types of variants is not performed.

The repeat-primed PCR test is used as a screening method for the presence or absence of a pathogenic CAG trinucleotide repeat expansion. This test can determine the number of CAG repeats on both patient alleles, within the tolerance limits specified by the ACMG (Bean & Bayrak-Toydemir 2014).

The fluorescent fragment-length assay is used to confirm the results from the repeat-primed PCR test (Jama et al. 2013.). However, this assay alone may not allow us to distinguish between individuals homozygous for a normal allele from those who are heterozygous for a normal allele and an allele with a large pathogenic expansion.

The results of both assays are necessary to screen patients for the presence or absence of the pathogenic CAG trinucleotide repeat expansion.

Alleses with greater than 39 repeats have been defined as pathogenic with full penetrance while alleles with fewer than 27 repeats have been defined as normal. Intermediate alleles of 27-35 repeats are considered normal mutable and alleles with 36-39 repeats have been defined as pathogenic with reduced penetrance (Bean & Bayrak-Toydemir 2014). While we do not know the specific upper limit of detection of an expanded allele using our assay methods, we have been able to detect an expanded allele containing ~180 repeats. It is expected that even with expanded alleles greater than this size we will be able to detect the presence of a large, pathogenic expansion. However, we may not be able to provide accurate sizing information beyond 180 repeats.

In patients where our test method did not reveal any variation between the two alleles, we cannot be certain that we were able to PCR amplify both of the patient's alleles. Occasionally, a patient may carry an allele which does not amplify, due for example to a deletion or a large insertion. In these cases, the report contains no information about the second allele.

The data that we report are based on DNA isolated from lymphocytes (or occasionally from buccal cells, or saliva). We can say nothing about the status of this CAG trinucleotide repeat in other tissues.

We have confidence in our ability to track a specimen once it has been received by PreventionGenetics. However, we take no responsibility for any specimen labeling errors that occur before the sample arrives at PreventionGenetics.

Interpretation of the test results is limited by the information that is currently available. Better interpretation should be possible in the future as more data and knowledge about the human genome and this specific disorder are accumulated.

**COMMENTS:** These results should be used in the context of available clinical findings, and should not be used as the sole basis for treatment. This test was developed and its performance characteristics determined by the PreventionGenetics Molecular Diagnostics Laboratories as required by the CLIA '88 regulations. It has not been cleared or approved for specific uses by the US Food and Drug Administration. The FDA has determined that such clearance or approval is not necessary. This test is used for clinical purposes. It should not be regarded as investigational or for research.
**SUPPLEMENTAL INFORMATION  V.17.03**

**SCREENING FOR THE HTT CAG TRINUCLEOTIDE REPEAT EXPANSION**

**Clinical Features:** Huntington disease is a neurodegenerative disease characterized by atrophy of the caudate nucleus and the putamen which leads to involuntary movements (chorea), progressive dementia, and psychiatric disturbances (Hayden and Kremer 2014). The average age of onset for Huntington disease is 40 years, but can range from late teens (juvenile onset) to over 60 years. Survival after the appearance of symptoms averages 18 years (Warby et al. 2014).

Development of symptoms progresses slowly, and is difficult to identify at times. The first signs to appear are a general slowing of intellectual ability, and a small personality change (Hayden and Kremer 2014). During this time, Huntington disease cannot be diagnosed based upon symptoms alone. During this period, PET scans can reveal decreased glucose metabolism in the caudate (Hayden and Kremer 2014). These symptoms eventually lead to the major signs of the disease: chorea, hypokinesia, rigidity, and dystonia. Chorea symptoms are defining to the disease. It is characterized by involuntary muscle movements, and they increase in severity throughout the course of the disease (Hayden and Kremer 2014). Gait disturbances, global cognitive decline, and dysphagia are also common as the disease progresses (Hayden and Kremer 2014).

There are currently no long term treatment options to slow disease progression. Treatment at this time is limited to palliative care, attempting to address the most serious symptoms. RNA interference studies are currently being conducted as a possible treatment option for Huntington disease (Fiszer et al. 2013).

**Genetics:** Huntington disease is inherited in an autosomal dominant manner. It is caused by a CAG repeat expansion in the *HTT* gene which occurs in the first exon, and encodes a polyglutamine tract beginning at residue 18. Repeat copy numbers can be categorized into 4 different categories: <27 repeats – normal, 27-35 – normal mutable, 36-39 – reduced penetrance, >39 full penetrance Huntington disease (Jama et al. 2013).

Typically, the more repeats in an individual, the earlier symptoms will develop. The largest repeats, ranging above 60 repeats to around 250 repeats (Bean & Bayrak-Toydemir, 2014) are causative of juvenile-onset Huntington disease (Warby et al. 2014). Huntington Disease affects 3-7 individuals per 100,000 in populations of European decent (Milunsky et al. 2003). Some regions have markedly lower rates of Huntington disease (Japan 0.38 per 100,000; African descent 0.06 per 100,000) while certain European regions have significantly higher prevalence (North Sweden 144 per 100,000; Moray Firth [Scotland] 560 per 100,000) (Hayden and Kremer 2014).

Huntington disease does not necessarily follow classical Mendelian inheritance patterns. For example, larger repeat tracts are more unstable and prone to undergo expansions or contractions (Semaka et al. 2013). Anticipation, or the increase of severity of a disease over successive generations, has been widely documented (Warby et al. 2014). According to Semaka et al. (2006), 80% of juvenile onset cases (large expansions) occur on the paternally inherited allele. Mosaicism of the *HTT*-CAG repeat has been reported and seems to be more prominent in juvenile-onset cases. However, according to the ACMG, the degree of mosaicism is not substantial enough to affect the interpretation of results obtained from peripheral blood (Bean et al. 2014; Telenius et al. 1994).

The *HTT* (huntingtin) gene (4p16.3) contains 67 exons and encodes the 348kDa huntingtin protein (Hayden and Kremer 2014). The exact function of the huntingtin protein is unknown (Zuccato et al. 2010). The polyglutamine region has been shown to be an essential regulator for binding partners. The conformation of this region is potentially flexible, allowing for the interactions with a multitude of other proteins (Kim et al. 2009). It has also been proposed that the polyglutamine tract plays an important role in protein aggregation, and possibly aids in escaping protein degradation (Hayden and Kremer 2014).
Testing Strategy: This test consists of a combination of two complementary analyses: (1) a repeat-primed PCR assay, and (2) a fluorescent fragment-length assay.

Repeat-primed PCR Assay
The repeat-primed PCR assay is used to determine the presence or absence of a nucleotide repeat expansion, as previously described (Warner et al. 1996; Kalman et al. 2007; Jama et al. 2013). PCR is performed with a fluorescently labeled forward primer specific to the HTT locus. The reverse primer anneals to multiple locations within the HTT repeat region. Due to the multiple annealing sites, amplicons will vary in size according to the number of repeats. PCR products are then analyzed on an ABI3730xl sequencer.

Fluorescent Fragment-length Assay
PCR analysis using primers that flank the HTT-CAG repeat allows full length amplification of both normal and expanded alleles. The purpose of this assay is to confirm the results obtained from the repeat primed PCR assay, as well as to help distinguish between individuals homozygous for an allele versus those with one normal sized allele and a second allele containing a large expansion. This procedure also helps to confirm allele sizes in individuals who are heterozygous for two alleles that are very close in repeat number.

Expanded repeats have been known to fail to amplify due to their large size. At this time, expansions of up to ~250 repeats (Bean & Bayrak-Toydemir) have been reported in affected individuals. Therefore in affected individuals carrying such large repeats, PCR amplification using specific primers that flank the CAG repeat sequence may result in the amplification of only the smaller sized allele. Of note, we have successfully visualized a sample with an allele of approximately 180 repeats using this method.

Indications for Testing: Testing should follow the guidelines as proposed by the ACMG. All patients with symptoms suggestive of Huntington disease are candidates for this test. This test is also recommended for patients with a familiar history of Huntington disease. Predictive testing is not recommended for patients under the age of 18 (to allow for a patient’s own, informed decision) due to the debilitating nature of the disease, lack of treatment options (Bean & Bayrak-Toydemir 2014), and the potential psychological issues that may result from either a positive or negative test result (Semaka et al 2006). Testing can also be useful for family planning decisions.

Sensitivity of Test: This test is designed to identify the number of CAG repeats in the two alleles for HTT. The test will determine the number of repeats within the accuracy guidelines of the ACMG (as listed below) (Bean & Bayrak-Toydemir 2014).

Number of CAG Repeats Tolerance
- <50 ±2 Repeats
- 50-75 ±3 Repeats
- >75 ±4 Repeats

Clinical sensitivity is nearly 100% when a patient presents with symptoms and a familial history of the disease (Saft et al. 2013). Up to 25% of HD patients may present with symptoms without familial history (Saft et al., 2013).

Targeted mutation analysis, through the combination of the two gene-centered PCR methods (repeat primed and fragment assays) of the HTT/CAG repeat region, is predicted to have a nearly 100% detection rate for pathogenic variants (Warby et al. 2014). There is a chance that extreme expansions could be missed with the fragment analysis, but the repeat primed PCR expansion assay should identify even extreme expansions.

References: Please see the test description for this test on our website (www.preventiongenetics.com) for a full list of complete citations.