

1 **Best Practice Guidelines for Molecular Analysis of Huntington Disease**

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14 These draft guidelines were fully updated and published for consultation in April 2010

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31 Keywords: Huntington Disease, genetic testing

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33
34 **Abbreviations**

35 DRPLA: Dentatorubral-pallidoluysian atrophy
36 ECACC: European Collection of Cell Cultures
37 EMQN: European Molecular Genetic Quality Network
38 EQA: External Quality Assessment
39 HD: Huntington Disease
40 HDL: Huntington Disease Like
41 HGVS: Human Genome Variation Society
42 *HTT*: Huntingtin gene
43 IA: Intermediate alleles
44 OECD: Organization for Economic co-operation and development
45 SCA: Spinocerebellar ataxia
46 STR: Short tandem repeat
47

48 **Description of the disease**

49 Huntington Disease (HD, OMIM #143100) is a progressive neurodegenerative disorder that
50 presents with motor symptoms, cognitive impairment and psychiatric disturbances. The first
51 symptoms usually manifest between 35 and 50 years of age and the duration of the disease
52 is between 15 and 20 years (Bates G 2002), (Pagon et al. 2002). About 5-10% of cases
53 present before the age of 20 (juvenile onset) and about 20% of cases present after 50 years
54 of age. HD is inherited in an autosomal dominant fashion with an incidence of 3-10 in 100 000
55 in populations of Western European descent. It is much less frequent (0.1-0.4: 100 000) in
56 other populations (Bates G 2002).

57
58 HD is characterized pathologically by loss of specific neuronal populations in many brain
59 regions although the pathology is not limited to neurons. Neuropathological features include
60 selective degeneration of neurons in the caudate and putamen and less severe loss in the
61 cerebral cortex. The preferential degeneration of medium sized spiny, enkephalin-containing
62 neurons of the indirect pathway of movement control in the basal ganglia provides the
63 neurological basis for chorea. Intraneuronal inclusions are a prominent feature of the disease
64 but are not a primary cause of pathology (Hackam, Wellington, and Hayden 1998).

65
66 Individuals homozygous for HD expansions appear to have a similar age of onset, but may
67 exhibit an accelerated rate of disease progression (Alonso et al. 2002).

68
69
70 **The gene and the mutations**

71 The gene involved, the huntingtin (*HTT*) gene (NM_002111; NC_000004) previously also
72 known as IT15, is located on chromosome 4p16.3, spans 180kb and consists of 67 exons.
73 The *HTT* gene is widely expressed and is required for normal development. It is expressed as
74 2 alternatively polyadenylated forms displaying different relative abundance in various fetal
75 and adult tissues. The larger transcript is approximately 13.7kb and is expressed
76 predominantly in adult and fetal brain, whereas the smaller transcript of approximately 10.3kb
77 is more widely expressed (Pruitt, Tatusova, and Maglott 2003).

78
79 Huntington Disease is caused by the expansion of an unstable polymorphic trinucleotide
80 (CAG_n) repeat in exon 1 of the *HTT* gene, which translates in an extend polyglutamine tract in
81 the protein. Alleles with less than 27 CAG repeats are classified as normal, while alleles with
82 36 or more repeats are detected in patients (see Table 1). Alleles with 27-35 repeats (called
83 mutable normal or intermediate alleles) are, in the vast majority of cases, not associated with
84 disease symptoms, but can expand into the affected range upon (predominantly paternal)
85 germline transmission and thus cause HD in offspring. Repeats of 36-39 CAG are
86 incompletely penetrant and can be found in affected individuals as well as individuals who
87 show no clinical symptoms in an advanced age (70 to 80 years and over). The CAG repeat
88 number in patients correlates inversely with age of onset of symptoms. Generally, individuals
89 with longer CAG repeats have an earlier age of onset. This is supported by findings that
90 individuals with very large CAG repeats (>65) present with juvenile HD, and individuals with
91 shorter CAG repeats (36-39) can remain asymptomatic. However, the number of repeats
92 accounts only for approximately 70% of the variance in age at onset (Rubinsztein et al. 1993).
93 Numerous predicting models, discussing the statistical relationship between the CAG repeat
94 length and the age of onset have been published over the last 15 years. A review and
95 validation study of these statistical approaches can be found in Langbehn et al. 2004.

96
97 Huntingtin is a protein of 3144 amino acids with a predicted molecular mass of 348kD. The
98 polyglutamine tract starts at residue 18 and, when abnormally expanded, is thought to acquire
99 a novel deleterious function. This eventually leads to neuronal dysfunction and
100 neurodegeneration. The polyglutamine expansions also result in the formation of neuronal
101 intranuclear inclusions containing huntingtin, ubiquitin and many other proteins.

102
103 **Reasons for referral**

104 Confirmation of a clinical diagnosis

105 This is usually requested by a neurologist or a clinical geneticist. Depending on local policies
106 other treating physicians might request a confirmation or exclusion of a clinical diagnosis of
107 HD. If the diagnosis has been confirmed by DNA analysis the patient and family members

108 should be referred for genetic counselling and presymptomatic or prenatal testing can be
109 offered. If available, preimplantation genetic diagnosis can be offered to at risk couples. While
110 presymptomatic testing is usually not available for them, minors with symptoms of juvenile HD
111 can be offered diagnostic testing. One should bear in mind that, in case of a confirmation of
112 the diagnosis, also one of the parents will get a presymptomatic test result. A presymptomatic
113 test for the parent should be considered in parallel, and an appropriate supporting structure
114 for the family should be available.

115 116 Presymptomatic testing

117 According to the international guidelines, predictive testing for HD should only and exclusively
118 be requested by a clinical geneticist (Guidelines for the molecular genetics predictive test in
119 Huntington's disease. International Huntington Association (IHA) and the World Federation of
120 Neurology (WFN) Research Group on Huntington's Chorea 1994). Presymptomatic testing
121 can be offered to individuals at an *a priori* 50% or 25% risk (depending on their position in the
122 pedigree). In very rare occasions also an individual at 12.5% risk is tested. Confirmation of
123 HD at the molecular level in at least one family member with HD is advisable. Presymptomatic
124 testing of minors (<18 years of age) should be avoided (Guidelines for the molecular genetics
125 predictive test in Huntington's disease. International Huntington Association (IHA) and the
126 World Federation of Neurology (WFN) Research Group on Huntington's Chorea 1994).
127 Comprehensive genetic counselling is required, and informed consent must be provided in
128 accordance with local practices.

129 130 Prenatal testing and preimplantation genetic diagnosis:

131 Prenatal testing is usually requested by a clinical geneticist. The testing method is identical to
132 presymptomatic testing, but the maternal or both parental samples are analyzed in the same
133 run. If the fetal genotype is equal to the maternal genotype, maternal contamination should be
134 excluded using polymorphic microsatellites; where the prenatal test does not provide
135 evidence to exclude significant MCC, evidence from a minimum of 2 microsatellite markers is
136 required to report that significant MCC has been excluded.
137 Preimplantation genetic diagnosis is only offered in a limited number of specialized centres
138 and will not be discussed here.

139 140 The exclusion test

141 In cases where an individual with a 25% risk to develop HD requests predictive testing but the
142 parent, who has a 50% risk, does not want to be informed about his or her risk an exclusion
143 test can be offered. This test is nowadays almost exclusively used in prenatal testing where
144 the foetus is at 25% risk and the future parent at 50% risk. It is performed with linked
145 polymorphic markers (see also interpretation section).

146 147 148 Repeat size ranges

149 Normal alleles:

150 Alleles in the range of 6-26 CAG have never found to be associated with HD and have
151 seldom been described as unstable after transmission to the next generation (De Rooij et al.
152 1993). The CAG repeat is highly polymorphic in the population.

153 154 Disease size range:

155 The smallest number of CAG repeats described in patients with confirmed clinical features of
156 HD is 36 (Rubinsztein et al. 1996), (Brinkman et al. 1997). This repeat size has been
157 observed in more than 6 documented HD cases. Although it is possible that HD cases with
158 fewer repeats exist, only few (n=5) possibly affected cases with 29-34 repeats were reported
159 (Kenney, Powell, and Jankovic 2007, Groen et al. 2010, Andrich et al. 2008, Herishanu et al.
160 2009). Such cases are difficult to ascertain due to the paucity of alleles of this size. Although
161 HD may be associated with these repeat sizes, the published data are not conclusive, and
162 alternative diagnoses need to be carefully considered in such cases with HD-like signs.

163 164 Range of reduced penetrance:

165 Elderly asymptomatic individuals with HD alleles of 36-39 repeats have been reported
166 repetitively (Rubinsztein et al. 1996, Brinkman et al. 1997, McNeil et al. 1997, Quarrell et al.
167 2007), defining the range of incomplete or reduced penetrance of the mutation.

168

169 Intermediate or mutable normal alleles:

170 Inconsistencies and confusion exist in literature regarding intermediate alleles despite the
171 existence of published guidelines (Semaka et al. 2006, personal observations during External
172 Quality Assessment for EMQN). These alleles of 27-35 repeats have been designated
173 “intermediate” and are also referred to as “mutable normal” alleles. They are defined as
174 “being below the affected range but having the potential to expand into the disease range in
175 the next generation”. The lower limit is defined as the shortest size ever reported to expand
176 into the HD range after one transmission. Intermediate alleles are relatively common in the
177 general population with frequency estimates between 1 and 3.9% (Semaka et al. 2006).
178 The risk of expansion of an intermediate allele into the disease range has been estimated at
179 0.1 to ~5-10% per generation (Semaka et al. 2006), (Hendricks et al. 2009); see also next
180 paragraph). Individuals who are found to carry alleles in this range should be counselled
181 about the possibility of prenatal diagnosis and the risk for other family members. Where
182 intermediate and pathogenic alleles are found in the same individual, the interpretation should
183 clearly distinguish the risks associated with each allele.

184

185 Repeat instability

186 Although rare, new mutations in HD have been described and originate from intermediate
187 alleles of 27-35 repeats, mainly through paternal transmission (Goldberg et al. 1995, Myers
188 et al. 1993). Up to now, only one maternal case has been documented (MJ van Belzen et al.
189 2009). Factors influencing repeat instability include size of the allele, sex and age of the
190 transmitting parent, family history and gene sequence and haplotype on which the
191 intermediate allele is located. Alleles with larger number of CAG repeats have a higher
192 likelihood of expanding into the affected range upon transmission to the next generation
193 (Semaka et al. 2006). The risk that offspring will develop HD is highest for fathers older than
194 35 years of age. Intermediate alleles can be coincidentally identified when healthy family
195 members in an HD family undergo CAG repeat sizing. In contrast they can be ascertained
196 from new mutation families and these new mutation alleles are more prone to repeat
197 expansion when compared to similar sized alleles in the general population. The genetic
198 variability near the repeat tract can influence its stability. The delta 2642 polymorphism is
199 vastly overrepresented on HD chromosomes (38%) relative to the general population (7%).
200 Finally, repeat tracts in which the penultimate CAA repeat has changed to a CAG are
201 markedly more unstable. Both polymorphisms tend to cluster in specific haplotypes (Crawford
202 and Nickerson 2005). Additional information is needed to determine a more precise and
203 critical risk assessment for offspring of intermediate allele carriers.

204

205 Anticipation:

206 Anticipation is the phenomenon in which increasing disease severity and/or decreasing age of
207 onset is observed in successive generations. In HD, it occurs more commonly through
208 paternal transmission. The phenomenon arises due to the expansion of the unstable CAG
209 repeat during spermatogenesis. Also large expansions, e.g. an allele size increment of > 7
210 repeats, occur almost exclusively through paternal transmission and might result in juvenile
211 onset of HD in the next generation.

212

213

214 **Analytical methods**

215 Regardless of the PCR-based strategy selected, it is important that the assay conditions are
216 optimized to ensure the accurate and unambiguous determination of the number of repeats.

217

218 The number of repeats is established by PCR analysis of the region encompassing the CAG
219 repeat, usually followed by fragment sizing through capillary or gel electrophoresis at
220 sufficient resolution to allow separation of alleles with one repeat difference (Warner, Barron,
221 and Brock 1993, Andrew et al. 1994). Other methods with comparable resolution can also be
222 applied. Laboratories are expected routinely to measure allele sizes and calculate margins of
223 error for the purposes of internal quality control. PCR products containing CAG repeats
224 migrate anomalously in electrophoresis, making conventional size ladders are unreliable.
225 Instead, control samples with well defined repeat sizes, preferably determined by DNA
226 sequencing, can be used for allele sizing. A cell line (or DNA prepared from it) from a patient
227 shown by sequencing to carry 24 and 35 repeats is available from the ECACC (European

228 Collection of Cell Cultures) . A useful panel of well-characterized DNAs is available from
229 Coriell CDC Repository (Kalman et al. 2007).

230

231 It is the laboratory's responsibility to empirically determine the detection limits of their assay,
232 while participation in external quality assessment (EQA) scheme allows the comparison of
233 allele sizing with other laboratories. Acceptable error limits are ± 1 repeat for alleles ≤ 42 and ± 3
234 repeats for alleles >42 . The CAG repeat in the *HTT* gene is adjacent of a 5' positioned CCG
235 repeat which is also polymorphic in length (9, 10). The original primer sets, used to size the
236 CAG repeats, also included this CCG polymorphism and might thus result in misclassification
237 of alleles (The Huntington's Disease Collaborative Research Group 1993). Therefore, this
238 assay should not be used for routine sizing of the CAG repeats. However, this assay can be
239 used to resolve two homozygous normal HD alleles with identical numbers of CAG repeats
240 but different numbers of CCG repeats.

241 To exclude the presence of a very large expansion that would be missed by PCR in case of
242 homozygosity of a normal CAG repeat allele, the use a Southern blotting protocol (PstI
243 digested DNA probed with Probe 4G6P1.7, available from Gill Bates) or TP-PCR (26) is
244 recommended (Warner et al. 1996). PCR of large (>100) CAG repeat tracks in DNA from
245 fresh or frozen tissue samples is feasible (Maat-Schieman et al. 2007). Since individuals with
246 more than 65 repeats will present with a juvenile or early onset form of HD, it will be unlikely
247 that these cases are missed by PCR analysis alone.

248

249 For homozygous (normal) CAG-testing results, the several approaches may be taken:

- 250 1) As mentioned above, the CAG-CCG repeat should be genotyped which might
251 demonstrate the presence of two normal alleles (with identical CAG repeat counts but
252 heterozygosity for the CCG-repeat)
- 253 2) Samples homozygous for CAG and CCG repeats should be analyzed with
254 appropriate – large CAG-repeat - positive control (demonstrating the sensitivity of the
255 test for larger CAG expansions)
- 256 3) In a symptomatic test setting, the age at onset of symptoms should be available and
257 discussed as to whether juvenile HD can be excluded clinically. i.e. if the patient has
258 onset in their 40s or 50s, a very large expansion is most unlikely.
- 259 4) In a presymptomatic test setting, while the proband's age can be taken into account, it
260 may be advisable to offer genotyping for the proband's parents in order to confirm
261 homozygosity or to check allele segregation by STR analysis.
- 262 5) In case of juvenile HD setting, a homozygous (normal) CAG result has to be doubted
263 unless segregation analyses with polymorphic markers has shown the presence of
264 two normal alleles. If parental samples are not available, Southern blotting or TP-PCR
265 should be considered.

266

267 The use of a no-template blank and a large expansion control with each batch of samples
268 tested is a requirement. Controls of known repeat number are also required, for accurate
269 sizing of patient samples. In addition, controls at the borders of normal-intermediate;
270 intermediate-reduced penetrance and reduced penetrance-affected ranges can be included in
271 the assay to improve the accuracy of repeat sizing in these areas and allele classification.

272

273 Three very rare polymorphisms have been described in the 3' or 5' primer used in some CAG
274 specific assays (Gellera et al. 1996, Margolis et al. 1999). These can potentially disrupt primer
275 binding to an HD chromosome and result in the failure to amplify a pathogenic allele (Gellera
276 et al. 1996). This can be checked in apparently normal homozygous cases by using additional
277 assays with an alternative downstream primer, e.g. one which includes the CCG repeats,
278 and/or amplifying only the CCG repeat (Gellera et al. 1996, Margolis et al. 1999) or excluding
279 a large expansion by Southern blot or TP-PCR.

280

281 Finally, in some countries it is common practice for presymptomatic tests to ask for a second
282 independent blood sample to confirm the result of the first analysis.

283

284 **Diagnostic sensitivity and specificity**

285 The absence of HD pathology in an individual with ≥ 40 repeats who died after living up to or
286 past normal life expectancy has never been described. Therefore a result of ≥ 40 repeats is

287 100% specific (Potter, Spector, and Prior 2004). CAG-repeat expansions account for >99% of
 288 cases of HD and, therefore, the test is >99% sensitive (Potter, Spector, and Prior 2004).

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 290

291 **Interpretation of the results and reporting**

292 The interpretation is always done in the context of the clinical referral and is summarized in
 293 Table 1. It is important to make a clear distinction between a report for a diagnostic test on a
 294 patient with symptoms of HD and a report for presymptomatic testing. One has to bear in
 295 mind that the result is not only important for the consultand tested but is also relevant for the
 296 family members. Each laboratory has its own reporting format, but general reporting
 297 guidelines can be found on the EMQN website www.emqn.org. One should also adhere to the
 298 OECD guidelines (Organization for Economic co-operation and development guidelines for
 299 quality assurance in molecular genetic testing. 2009). A one page report is the preferred
 300 format in which the test result and the answer to the clinical question should be easy to find
 301 and unambiguously formulated. The report is a stand-alone document that should not only be
 302 clear to the referring physician but also to other professionals involved in supporting and/or
 303 treating the patient. The answer to the clinical question, the *take-home-message*, should be
 304 stated clearly and unambiguously: for example 'The diagnosis HD either *is* or *is not*
 305 confirmed.' 'The proband *will* or *will not* develop HD. Although local policy can vary with
 306 regard to reporting, some relevant HD specific items are mentioned below. Some sample
 307 paragraphs for reporting particular scenarios are included as an appendix to these guidelines.

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Table 1. Summary of the implications of different repeat ranges for the individual tested and family members.

Number of repeats	Implications for individual/patient		Implications for family members
	Diagnostic test	Predictive test	
9-26	Diagnosis not confirmed or Diagnosis of HD excluded	Will not develop HD	No increased risk for HD
27-35	Diagnosis not confirmed or Diagnosis of HD excluded	Will not develop HD	Increased risk for HD (few %; <<10%)
36-39	Diagnosis of HD confirmed	May develop HD but in range of reduced penetrance	Increased risk for HD
40+	Diagnosis of HD confirmed	Will develop HD	Increased risk for HD

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 315

316 Nomenclature:

317 The number of repeats is measured in relation to the *number of uninterrupted CAG repeats*.
 318 There are more glutamines than CAG repeats in this part of the gene, as the polyglutamine
 319 tract is coded for in most chromosomes by (CAG)_nCAACAG (CAA also codes for a
 320 glutamine). The HGVS nomenclature is not considered the most appropriate for reporting the
 321 results of trinucleotide repeat analysis. Instead, the number of repeats is used as a result of
 322 the genetic test, and the error limits of the test are always mentioned. Reporting actual allele
 323 sizes is subject to local practice. A statement whether the allele is in the normal or the
 324 disease range can very well be sufficient. However, it is important to report actual allele sizes
 325 if this is relevant for the interpretation of the result; for instance in case of a reduced
 326 penetrance or intermediate allele.

327

328 Diagnostic testing:

329 In case of a CAG-repeat ≥36 the diagnosis HD is confirmed (*or* is consistent with a diagnosis
 330 of HD). In case of a CAG-repeat ≤35 the diagnosis is excluded (*or* the result is not consistent
 331 with HD). However, recently a few possibly affected cases with 29-34 repeats were reported
 332 (Kenney, Powell, and Jankovic 2007, Groen et al. 2010, Andrich et al. 2008, Herishanu et al.
 333 2009). Implications of alleles in the reduced penetrance or intermediate (normal mutable)

334 range should be discussed in the report. In case of a confirmation of HD, the family should be
 335 offered a referral for genetic counselling.
 336

337 In case of a test result with two alleles in the normal range when disease symptoms are
 338 clearly present, the referring physician could consider testing for HD-like disease, like HDL1
 339 (PRNP gene), HDL2 (JPH3 gene), DRPLA (ATN1 gene) or SCA17. This can be mentioned in
 340 the report in more general terms.
 341

342 Predictive (presymptomatic) testing:

343 In cases of a CAG-repeat from 36 to 39 the individual is at risk of developing HD; in cases
 344 with a CAG repeat ≥ 40 the individual will develop HD (*or* is at risk of developing HD). In cases
 345 of a CAG-repeat ≤ 35 the individual will not develop HD (*or* is not at risk of developing HD).
 346 Implications of alleles in the reduced penetrance or intermediate (normal mutable) range
 347 should be discussed in the report. If appropriate, a prenatal test can be offered.
 348

349 Data in which the likelihood that an individual with a particular size CAG repeat will be
 350 affected by a specific age have been published (Langbehn et al. 2004). However, extreme
 351 care should be taken applying this information in individual presymptomatic cases.
 352

353 Exclusion test:

354 The test result reveals whether the individual with an a priori 25% risk received the grand-
 355 parental risk haplotype or not (see also reason for referral). If the grand-parental risk
 356 haplotype is present, the risk for this individual is increased to 50%. If it is not present the
 357 individual's risk is reduced to 0%. It is necessary to include DNA of at least one grandparent
 358 in the analysis. Confirmation of the diagnosis at the molecular level in at least one family
 359 member, preferably the grandparent with HD, is necessary. For the exclusion test
 360 polymorphic markers in the *HTT* region on chromosome 4p16.3 can be used (see Table 2 for
 361 a list of STRs). Ideally, markers both proximal and distal to *HTT* need to be informative to
 362 have a secure result. It is advisable to determine beforehand which markers are informative
 363 for the particular family under study. A pedigree with the result of the haplotype analysis
 364 should be included in the report.
 365

366 Some paragraphs adjudged by EQA assessors to meet all the requirements for
 367 comprehensiveness and clarity are attached as Appendix 1

Comment: Does anyone have a suggestion for a nicer end to the story?

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Table 2. Suggested markers in 4p16.3 that are suitable for exclusion testing

Marker	UniSTS	Ensembl marker	Ensembl location	Het	Alias (Ref)
D4S2936	24920	Z52740	692247-692420		
D4S3038	42100	Z51777	1099931-1100155		
D4S1614	27925	Z24429	2646689-2646866		
D4S43	147240	D4S43	2336363-2336628	0.7	C39 (Tagle, Blanchard-McQuate, and Collins 1992)
D4S127	149984	D4S127	3038714-3038864	0.7	P363 (Taylor et al. 1992)
HTT			3076408-3245687		
D4S3034	38369	Z51717	3325536-3325722	0.6	
D4S412	9920	Z16836	3380781-3380974		
D4S2957	73817	Z53093	3833487-3833597	0.6	
D4S431	14923	Z17175	6415645-6415795		

374

DRAFT

376 **Appendix: Examples of text that can be used in the interpretation section of a report:**

377 The text comes from reports submitted by participants in EQA schemes organized by the EMQN

378

379 Example 1 predictive test with result of 17 and 36 CAG repeats in a 62-year old female

380

381 *"The patient has an expansion which is associated with Huntington Disease but has reduced*
382 *penetrance. An allele with 36 CAG repeats can cause clinical symptoms typical for HD but the*
383 *patient may also remain asymptomatic. There is a possibility that the repeat size increases*
384 *after transmission to the subsequent generation. This is less likely when this allele is*
385 *transmitted via the female germline. Her children may nevertheless be at risk of having*
386 *inherited an expanded HD allele. It is advisable to refer for genetic counselling."*

387

388 *"... besides a non-pathological repeat with 17 repeats a 36 repeat allele was detected. Since*
389 *the 36 repeat allele lies within the range of reduced penetrance, it is not possible to predict*
390 *with certainty whether she will be affected later in life."*

391

392 *"...in the range of reduced penetrance (36-39 repeats). Based on the current knowledge it is*
393 *not possible to predict with certainty for this individual whether she will develop HD or not."*

394

395 Example 2 predictive test with result of 31 and 35 CAG repeats in a 28-year old female

396

397 *"...presymptomatic testing for HD indicates it is most unlikely that she will develop HD.*
398 *However, counselling for this patient, her family and (future) offspring is rather complex. In*
399 *subsequent generations, there is an as yet undetermined risk of expansion into the disease*
400 *size range for both intermediate alleles. "*

401

402 *"...as she carries 2 intermediate alleles, she will not develop HD. However, by transmission of*
403 *these alleles to the next generation, they may expand into the disease causing range.*
404 *Therefore, each of her (future) children is an obligate carrier and they have an increased risk,*
405 *especially after paternal transmission, to have offspring affected with HD."*

406 Internet resources:

407

408 - EMQN website www.emqn.org

409 - GeneReviews funded by NIH. Developed at the University of Washington, Seattle,
410 USA <http://www.ncbi.nlm.nih.gov/bookshelf/br.fcgi?book=gene&part=huntington>

411 - NCBI RefSeq Gene project ftp://ftp.ncbi.nih.gov/refseq/H_sapiens/RefSeqGene/

412 - European Collection of Cell Cultures <http://www.hpacultures.org.uk> Cell line number
413 CM0034, ECACC Ref. No: 95090133.

414 - CDC Centers for Disease Control and prevention: Cell and DNA repository
415 <http://ccr.coriell.org/sections/collections/>

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