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Report

A Recurrent Expansion of a Maternal Allele with 36 CAG Repeats Causes Huntington Disease in Two Sisters

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Large intergenerational repeat expansions of the CAG trinucleotide repeat in the HD gene have been well documented for the male germline. We describe a recurrent large expansion of a maternal allele with 36 CAG repeats (to 66 and 57 repeats, respectively, in two daughters) associated with onset of Huntington disease (HD) in the second and third decade in a family without history of HD. Our findings give evidence of a gonadal mosaicism in the unaffected mother. We hypothesize that large expansions also occur in the female germline and that a negative selection of oocytes with long repeats might explain the different instability behavior of the male and the female germlines.

Huntington disease (HD) is a progressive neurodegenerative disease. The disease is characterized by involuntary movements and cognitive and psychiatric disturbances caused by the neurodegeneration of the basal ganglia and cerebral cortex. The HD gene belongs to a group of genes causing neurodegenerative diseases through the expansion of a polymorphic CAG repeat (The Huntington's Disease Collaborative Research Group 1993). According to the American College of Medical Genetics and of the American Society of Human Genetics (ACMG/ASHG), the range of mutated alleles spans 40–250 CAG repeats (ACMG/ASHG statement 1998), whereas nonexpanded alleles carry <27 CAG repeats. Alleles of 27–35 repeats have not until now been associated with HD. The ACMG/ASHG statement suggests that these alleles be called "mutable normal alleles" because of their potential meiotic instability (Goldberg et al. 1993; Myers et al. 1993). Alleles with 36–39 CAG repeats should be considered as having a reduced penetrance. So far, large intergenerational expansions of >20 CAG repeats that are maternally transmitted have seldom been described. In a recent report (Sanchez et al. 1997), there is evidence of a moderate expansion through the maternal germline from 37 to 43 CAG repeats. Nance et al. (1999) described a juvenile form of HD in a child with >200 CAG repeats that probably originated from a maternal allele of 60 CAG repeats.

We have investigated two sisters (from a group of seven siblings) with suspected HD and no family history of HD. The first proband was 33 years old at the time of the molecular test. Her clinical symptoms were: paralysis of horizontal ocular movements, very serious dysarthria, severe limb hyperkinesia, ataxic gait, intermittent athetosis, and increased reflex. The computer tomography provided evidence for a serious periventricular and cortical brain atrophy. Initial symptoms (impairment of comprehension, reduced memory, reduction of affective reactions, and reduction of spontaneous activity) had been observed by the parents around age 16–17 years. The younger sister (30 years old) shows a serious dysarthria, hyperkinesia of the limbs and face, ataxic gait, and impaired coordination. Her emotional state is often inappropriate, with unrealistic future expectations. The first neurological signs were seen at age 23 years as athetosis and gait impairment. The parents reported that gait disturbance had been apparent for a long time. The diagnosis of HD was delayed by the absence of a positive family history. DNA of the probands was extracted using a standard procedure. PCR amplifications were carried out as described elsewhere (Warner et al. 1993). Analysis of the CAG trinucleotide of the HD gene revealed the presence of 66 CAG repeats in the older sister and 57 CAG repeats in the second

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sister (fig. 1B). PCR of the DNA of the father amplified normal alleles with 20 and 21 CAG repeats. Interestingly, analysis of the maternal CAG repeats detected an allele with 36 CAG repeats and a normal allele with 18 CAG repeats. The 53-year-old mother did not have any clinical signs of HD. PCR-mediated analysis of the loci D4S115, D4S126, and D4S2936 and of the adjacent CCG triplets in the HD gene demonstrated that both sisters had inherited the same maternal chromosome 4 (fig. 1A). The maternal allele with 36 CAG repeats expanded in both cases by >20 repeats. To exclude a general instability of CAG repeats, we analyzed the SCA1 gene, the Machado-Joseph–disease gene, and the dentato-rubral-pallidoluysian atrophy gene. All alleles were found to be stable and not expanded (genotyping available under request). The analysis of several polymorphic markers shows that the two sisters have different fathers (data not shown). The size determination of the CAG repeats of the mother and of the two sisters have been verified by sequence analysis of cloned PCR products. At the 3′ end of the CAG repeat, we did not find any sequence changes that have been previously described as factors inducing allele instability (Chong et al. 1997) (sequences provided, data not shown). Several studies have shown that expanded CAG alleles are unstable during transmission, particularly through the male germline. The instability of CAG repeats in the HD gene has been directly demonstrated with PCR-based analysis of sperm population and single sperm showing a high variability of HD alleles (Telenius et al. 1994; Goldberg et al. 1995; Telenius et al. 1995; Chong et al. 1997; Leeflang et al. 1999). As a matter of fact, the sex of the transmitting

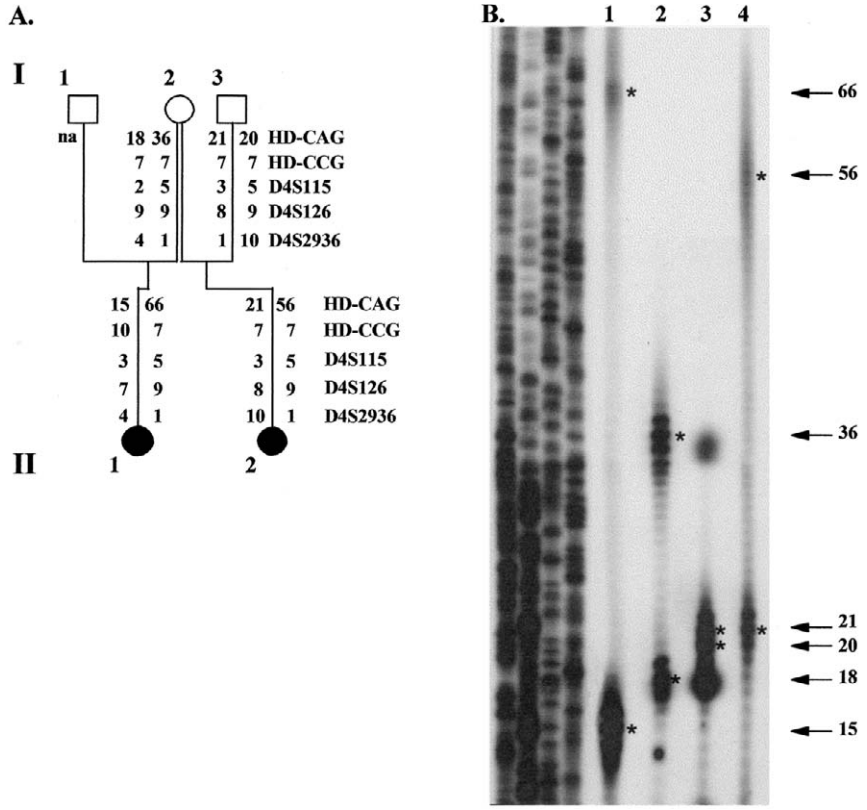

Figure 1 *A,* Pedigree of the family showing the genotype of the family. The two affected sisters harbor expanded alleles of 66 CAG repeats and 57 CAG repeats. The allele of the proband II1, with 15 CAG repeats and 10 CCG repeats, must be of paternal origin, since the mother (I1) does not carry any of them. Therefore, the allele with 66 CAG repeats is of maternal origin. The second proband (II2) carries an allele with 21 CAG repeats inherited from the father (I2) and, consequently, the expanded allele with 57 CAG repeats inherited from her mother. The analysis at locus D4S115 proves that the sisters inherited the same maternal chromosome 4 from the mother and that the genotyping at locus D4S2936 is compatible with the inheritance of the same maternal chromosome 4. The genotyping at locus D4S126 was not informative. *B,* Radiograph of the PCR analysis of the CAG repeats in the HD gene. Genomic blood DNA was amplified from two sisters and the parents, as described, and the products were separated on a 5% denaturing polyacrylamide gel. The father of II1 (lane 2) carries 20 and 21 CAG repeats. The mother (lane 3) has one allele with 18 CAG repeats and a second allele in the "mutable normal allele" range of 36 CAG repeats. In lane 1 is the large allele of ~66 CAG repeats and the small one of 15 CAG repeats. In lane 4, the expanded allele is of ~57 CAG repeats and the small of 21 CAG repeats. The marker is on the left (M13mp18). Asterisks (*) denote CAG alleles.

parent seems to be one of the most important elements influencing the stability of expanded alleles and of HD alleles (Leeflang et al. 1995). The difference in expansion behavior of HD alleles in men versus that in women might suggest sex-specific effects of gametogenesis on the intergenerational CAG-repeat stability. Small slippages of DNA polymerase at first might gradually lead to an expansion, until a critical number of repeats is reached, which could then undergo large expansions by hairpin formation. This expansion mechanism might be more "efficient" in the male germline because of continuous cell division of spermatogonia throughout adult life. This suggestion might also explain the tendency for repeat length in the offspring to increase as a function of the paternal age (Farrer et al. 1992). To date, however, there is no convincing explanation for the sex-specific differences in stability. Our finding clearly demonstrates the possibility of large expansions through the female germline, most probably, in our case, as a result of gonadal mosaicism. Recent findings about the pathological mechanism of polyglutamine expansions indicate a toxic effect of the mutated proteins on specific cell populations (Davies et al. 1997; Saudou et al. 1998). We hypothesize that expansions and contractions of "unstable" CAG repeats might occur in both genders during embryonic development. Up to puberty, all primary oocytes are arrested in the first meiotic prophase until maturation. We suggest that in disorders where the protein is expressed in oocytes (Li et al. 1993), as is the case with huntingtin, the primary oocytes undergo progressive cell death during this resting period, and those with the longest polyglutamine tracts are eliminated first, causing a relative enrichment of oocytes with small CAG repeats. The probability of transmission of larger CAG repeats by women carrying a CAG expansion could inversely correlate with maternal age at conception. The corollary of this is that older pregnant women might have a greater probability of transmitting contracted repeats. In our case, the mother of the two patients was 20 and 23 years old at conception of the two daughters, which are relatively young ages. In accordance with the suggested model, the first sister has a larger expansion than the second (66 versus 57 repeats, respectively). The other five siblings, who were born later, did not have any clinical signs. Unfortunately, they were not available for a genetic test. Recently, Sato and colleagues examined the intergenerational changes of expanded CAG repeats in patients with dentato rubral-pallidoluysian atrophy (DRPLA) (Sato et al. 1999). They found an age-dependent increase in the number of CAG repeats among male transmitters. Conversely, there was an age-dependent tendency to contraction among female transmitters. Notably, the transmission through the female germline of alleles of increased size in offspring was restricted to young women. They also found similar patterns of instability in DRPLA transgenic mouse lines. Three further works (Kaytor et al. 1997; Wheeler et al. 1999; Shelbourne et al. 1999) have reported an instability of CAG repeats in transgenic/knock-in mice and describe similar contractions in female transmitters. These findings could be in accordance with a negative selection of oocytes with large expansions. A wide analysis of the age-related intergenerational changes of CAG repeats in the HD gene will be necessary to verify our hypothesis. In summary, our findings demonstrate the presence of gonadal mosaicism in the ovary and show that very large expansions are possible through the female germline that cause an early age at onset of HD.

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References

The American College of Medical Genetics/American Society of Human Genetics Huntington Disease Genetic Testing Working Group (1998) ACMG/ASHG statement: laboratory guidelines for Huntington disease genetic testing. Am J Hum Genet 62:1243–1247

The Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell 72:971–983

Chong SS, Almqvist E, Telenius H, LaTray L, Nichol K, Bourdelat-Parks B, Goldberg YP, et al (1997) Contribution of DNA sequence and CAG size to mutation frequencies of intermediate alleles for Huntington disease: evidence from single sperm analyses. Hum Mol Genet 6:301–309

Davies SW, Turmaine M, Cozens BA, DiFiglia M, Sharp AH, Ross CA, Scherzinger E, et al (1997) Formation of neuronal intranuclear inclusions underlies the neurological dysfunction in mice transgenic for the HD mutation. Cell 90: 537–548

Farrer LA, Cupples LA, Kiely DK, Conneally PM, Myers RH (1992) Inverse relationship between age at onset of Huntington disease and paternal age suggests involvement of genetic imprinting. Am J Hum Genet 50:528–535

Goldberg YP, Kremer B, Andrew SE, Theilmann J, Graham RK, Squitieri F, Telenius H, et al (1993) Molecular analysis of new mutations for Huntington's disease: intermediate alleles and sex of origin effects. Nat Genet 5:174–179

Goldberg YP, McMurray CT, Zeisler J, Almqvist E, Sillence D, Richards F, Gacy AM, et al (1995) Increased instability of intermediate alleles in families with sporadic Huntington disease compared to similar sized intermediate alleles in the general population. Hum Mol Genet 4:1911–1918

Kaytor MD, Burright EN, Duvick LA, Zoghbi HY, Orr HT

(1997) Increased trinucleotide repeat instability with advanced maternal age. Hum Mol Genet 6:2135–2139

Leeflang EP, Zhang L, Tavare S, Hubert R, Srinidhi J, MacDonald ME, Myers RH, et al (1995) Single sperm analysis of the trinucleotide repeats in the Huntington's disease gene: quantification of the mutation frequency spectrum. Hum Mol Genet 4:1519–1526

Li SH, Schilling G, Young WS 3d, Li XJ, Margolis RL, Stine OC, Wagster MV, et al (1993) Huntington's disease gene (IT15) is widely expressed in human and rat tissues. Neuron 11:985–993

Myers RH, MacDonald ME, Koroshetz WJ, Duyao MP, Ambrose CM, Taylor SA, Barnes G, et al (1993) De novo expansion of a (CAG)n repeat in sporadic Huntington's disease. Nat Genet 5:168–173

Nance MA, Mathias-Hagen V, Breningstall G, Wick MJ, McGlennen RC (1999) Analysis of a very large trinucleotide repeat in a patient with juvenile Huntington's disease. Neurology 52:392–394

Sanchez A, Mila M, Castellvi-Bel S, Rosich M, Jimenez D, Badenas C, Estivill X (1997) Maternal transmission in sporadic Huntington's disease. J Neurol Neurosurg Psychiatry 62:535–537

Sato T, Oyake M, Nakamura K, Nakao K, Fukusima Y, Onodera O, Igarashi S, et al (1999) Transgenic mice harboring a full-length human mutant DRPLA gene exhibit age-dependent intergenerational and somatic instabilities of CAG repeats comparable with those in DRPLA patients. Hum Mol Genet 8:99–106

Saudou F, Finkbeiner S, Devys D, Greenberg ME (1998) Huntingtin acts in the nucleus to induce apoptosis but death does not correlate with the formation of intranuclear inclusions. Cell 95:55–66

Shelbourne PF, Killeen N, Hevner RF, Johnston HM, Tecott L, Lewandoski M, Ennis M, (1999) A Huntington's disease CAG expansion at the murine Hdh locus is unstable and associated with behavioural abnormalities in mice. Hum Mol Genet 1999 8:763–774

Telenius H, Kremer B, Goldberg YP, Theilmann J, Andrew SE, Zeisler J, Adam S, et al (1994) Somatic and gonadal mosaicism of the Huntington disease gene CAG repeat in brain and sperm. Nat Genet 6:409–414

Telenius H, Almqvist E, Kremer B, Spence N, Squitieri F, Nichol K, Grandell U, et al (1995) Somatic mosaicism in sperm is associated with intergenerational (CAG)n changes in Huntington disease. Hum Mol Genet 4:189–195

Warner JP, Barron LH, Brock DJ (1993) A new polymerase chain reaction (PCR) assay for the trinucleotide repeat that is unstable and expanded on Huntington's disease chromosomes. Mol Cell Probes 7:235–239

Wheeler VC, Auerbach W, White JK, Srinidhi J, Auerbach A, Ryan A, Duyao MP, et al (1999) Length-dependent gametic CAG repeat instability in the Huntington's disease knock-in mouse. Hum Mol Genet 8:115–122