LETTERS TO THE EDITOR

A comparison of the Huntington’s disease associated trinucleotide repeat between Chinese and white populations

Huntington’s disease (HD) affects one in 10 000 people in most white populations. Its prevalence among the Chinese is not clear, but it is not expected to be much different from that of the white population. In 1993, a polymorphic (CAG)n trinucleotide repeat was identified1 in the 5’ region of a new transcript, IT15. The protein encoded by the IT15 was named huntingtin. The observation that the length of the trinucleotide repeat was increased in virtually all HD chromosomes examined,2,3 as well as in families with a presumed new mutation, suggests the mutational mechanism causing HD has been identified. The discovery of the mutation responsible for HD has important implications for both research and clinical practice. However, most of the data relate to white populations. In this study we verify the presence of the trinucleotide repeat elongation in Chinese patients with HD and establish the range of repeat sizes in affected and normal subjects. The diagnosis of HD was based on clinical and family histories, neurological examinations, and special investigations, such as electroencephalography and computerised tomography. Genomic DNA samples were available from a total of 114 persons (51 normal controls and 63 members of 11 unrelated HD families, including 11 unrelated spouses who also served as controls). The size of the (CAG)n trinucleotide repeat was analysed by the polymerase chain reaction (PCR). The genomic primer sequences used in our PCR assay for the (CAG)n repeat were HD-1: 5’ ATG AAG GCC TTC TGC CTC AAG TCC TTC 3’, and HD-3: 5’ GGC GGT GCC GGC TGT TGC TGC TGC TGC 3’. The PCR assay was performed as described by Warner et al with minor modifications. The PCR assay was highly specific and gave products in the 74 base pairs (bp) (nine CAG repeats) to 134 bp (29 CAG repeats) range from normal chromosomes, and products of 167 bp (40 repeats) and larger from HD chromosomes (figure). One normal and one expanded allele were found in all HD patients. The comparison of the size variation of triplet repeats in the control and HD chromosomes between Chinese and whites is shown in the table. Thirteen discrete allele sizes among the normal population, with a heterozygosity frequency of 80.7%, and an expanded range from 40 to 58 copies on HD chromosomes, were found. The peaks of the normal and HD ranges were well separated at 17 and 43 repeats, respectively. The overlap of the two distributions was observed (figure). Within our population, 65.7% of the expanded alleles fell in the range of 40 to 45 repeats, while only 17.1% consisted of more than 50 units.

This is the first study examining the HD mutation in a large Chinese population. The range of the CAG repeat length 59 expanded chromosomes (124 from 51 normal subjects and 11 unrelated spouses of the HD patients; 35 from the affected or symptomatic subjects, each having one chromosome that did not contain the expanded CAG repeat) in the Chinese population is much tighter (with a statistically significant smaller standard deviation) than the distributions reported in the United States3,4 and Europe5,6 (table). The distribution looks more like the recently published African and Japanese distributions where there is a lower incidence of HD. The median of the size of the upper alleles representing the expanded HD chromosomes was 44 repeats, with a range of 40 to 58 (figure, table). All Chinese patients in whom HD was diagnosed were heterozygous, with one allele in the normal range and one expanded allele, indicating that expansion of this (CAG)n repeat is the major cause of Huntington’s disease. A copy number of 40 or higher can result in the full phenotypic manifestations of the disease. Thus, mutual events leading to HD seem to be homogeneous worldwide.7,8 The finding that the molecular biology of HD is indistinguishable in Chinese and white populations is of great interest. The idea that worldwide HD evolved from a very limited number of European founders is no longer tenable.9,10 This study provides evidence that there is a basal rate of susceptibility to the HD mutation constantly adding new families. It will be very interesting to know whether new mutation rates differ between races.

MacMillan et al11 and Kremer et al12 reported an estimated sensitivity of 98% and a specificity of 100% for the use of the number of CAG repeats to identify those with the mutation for HD. The diagnostic value of the trinucleotide repeat expansion is apparently high within our population, with no overlap in values between normal and affected persons. This molecular test can be used to differentiate HD from other inherited movement disorders.11 We have not encountered any patient with the clinical phenotype of HD without the trinucleotide expansion. Moreover, the ability to measure the size of the trinucleotide repeat in people at risk for HD will revolutionise preclinical testing and eliminate the need for linkage analysis. This will greatly facilitate counselling and makes the test available to applicants without living relatives, and without undue involvement of other at risk sibs. We believe that the ethical and social issues involved will remain largely unchanged; they may well become more important as the
Rare variants of chromosome 9 with extra G positive band within the qh region are not alike

The intriguing findings in a recent investigation by Fernandez et al prompted us to re-evaluate our previous case1 that had a similar extra G positive band within the 9qh region by traditional molecular techniques. To our knowledge, these two studies, in addition to a third by Macera et al, are the only ones that have used various molecular techniques which showed satellite DNA locations in this particular so-called rare variant of chromosome 9. Many other reports which have focused on this seeming paradox, stated that the variant chromosome 9 appeared to be similar by conventional banding techniques. Nevertheless, a molecular approach using the FISH technique suggested that each of these three chromosome 9 variants apparently have different structural arrangements. These three plus approximately 100 other people who have had this variant are phenotypically normal.2 The structural organisation of the qh region in these rare variants have been shown by alpha, beta, and satellite III DNA probes by the FISH technique. In the study of Fernandez et al,3 the chromosome 9 displayed one alpha and one beta signal. Verma et al4 determined that a chromosome 9 had two alpha and two beta signals and their additional case5 showed that a chromosome 9 had one alpha and two beta signals which shed some light on the possible mechanism and origin of a variant of these so-called rare heteromorphisms.6 Three types of mechanisms of the origin of such heteromorphic variants have been described7 which can account for the alpha, beta, and satellite III DNA rearrangements within chromosome 9. Briefly, they are as follows. Type I: a break in each chromosome homologue is required, with one break on a chromosome at q21.2 and the other break in the homologue at or near the alpha/beta junction. Type II: initially, a pericentric inverted chromosome is essential with one break each at the p12/p13 interface and within the alpha satellite region; the satellite III/p13 border is the breakage site on the homologue. Type III: a break in the satellite III region of one chromosome and one break at band q21.2 on the homologue is necessary. Type I could account for the chromosome 9 in the report of Macera et al,8 type II for Verma’s group,9 and type III closely resembles the variant chromosome 9 of Fernandez et al.10

The extra band observed by Fernandez et al10 was resistant to AluI digestion and when introduced to 5-azacytidine treatment the band became homogeneously undecondensed. However, in the investigation of Verma et al the extra band was digested by AluI. All human centromeres contain alpha satellite DNA but not all centromeres are resistant to AluI treatment. This may be because of an evolutionary divergence at the DNA base pair level,11 but the reason why the G positive band in the case of Verma et al was not resistant to AluI digestion is apparently because of its euchromatic aetiology. The chromosome 9 from our previous case1 was subjected to 5-azacytidine incorporation and then examined by the FISH technique using a D9Z1 probe which detects classical satellite III DNA of chromosome 9 and a spectrum orange paint probe which detects the euchromatin of chromosome 9 (figs 1 and 2). Again, as in the AluI treatment, the result was the opposite of that of Fernandez et al and showed that the extra band was not affected and remained condensed. The induction of 5-azacytidine (3·5 × 10−4 M) in the last seven hours of incubation apparently hypomethylates the site replicating classical satellite constitutive heterochromatin regions of chromosomes 1, 9, 15, 16, and Y, and causes under-condensation owing to its incorporation during this period.12 It is believed the under-condensation of the heterochromatin that is caused by the induction of 5-azacytidine is possible owing to the relatively high variation in the number of repeated units within the classical satellite DNA region; generally this is not the case in the alpha and beta satellite DNA of chromosome 9.13 However, it has been shown that there are differences in the condensation inhibition behaviour of centromeric heterochromatin of chromosome 9 when treated with 5-azacytidine.7 As stated earlier14 the extra band in the variant chromosome 9 was “euchromatin in nature” and originated from chromosome 9, as indicated by the whole chromosome 9 painting probe (figs 1 and 2). Also, the fact that the extra band did not undercondense, as the heterochromatin did, suggests that this band is not late replicating and hence euchromatic in behaviour.

In the case of Fernandez et al,10 the extra band in the qh region of the variant chromosome 9 undercondensed owing to 5-azacytidine treatment. This may suggest that the band is late replicating and is not euchromatic in behaviour. In this case, the de-condensing effects of the 5-azacytidine treatment may have spread into euchromatic bands integrated into large blocks of classical satellite DNA. In contrast, the band material may be insensitive to decondensation if it is immediately juxtaposed with alpha or beta satellite DNA. The results from the AluI treatment also indicate the same properties. It is important to note the significant events which resulted in this rare chromosome 9 variant. The particular type of chromosome 9 that is involved in the rearrangement, the