

Huntington's disease in Malaysia: a clinical and genetic study

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Abstract

Huntington's disease (HD) is associated with an expanded (CAG) repeat within a novel gene 4p16.3(IT5). HD is one tenth less common in non-Caucasians. It has been hypothesised that HD in Chinese originated from a common Caucasian ancestry by way of migration with the opening the five port cities in China. A HD registry was established in Malaysia in 1995. In eighteen months we identified seven unrelated patients with HD. There were four Chinese, one Malay and two Indians. The CAG repeat ranges from 40-50. Only one Chinese family had possible Caucasian ancestry of Irish descent, but none of the patients or relatives has Caucasian features. The only Malay patient was a local and the two Indians had origins in Tamil Nadu and Punjab, India respectively. The genealogy of the Chinese patients were traced to small villages remote from the port cities. Culturally, Chinese women were forbidden to have close contact with foreigners. Additional genetic evidence show that the CCG repeat adjacent to IT5 is of seven repeats in Asians and 10 repeats in Caucasians. The distribution of the CAG repeats differ among populations of low prevalence and the west. Therefore, mutation of the IT5 gene rather than the European migration hypothesis is likely to be the explanation for the variation in prevalence of HD.

Key Words: Huntington's disease, Malaysia, Chinese, Indian, Malay

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant neurodegenerative disease characterised by progressive mental decline and chorea. HD is related to a mutation causing polymorphic trinucleotide (CAG) repeat on chromosome 4p16.3.¹ The detection of excessive trinucleotide repeats in the abnormal gene allows definitive diagnosis and pre symptomatic genetic testing in at-risk family members.

Malaysia is a multiracial country that has three main races. The Malays and other indigenous groups, Chinese and Indians make up approximately 62%, 29% and 8% of the population. In 1995 we established a nationwide HD Registry at the University of Malaya Medical Centre, Kuala Lumpur. The Medical Centre is one of the major public tertiary referral hospitals.

This is a report of the clinical and genetic studies of the patients seen since the setting up of the Registry. As many Malaysians are descendants of immigrants from China, India and Indonesia, we also traced the ancestral origins

of the affected families in order to establish any possible European origin of the disease.

MATERIALS AND METHODS

All patients referred with a clinical diagnosis of possible HD were assessed by a study neurologist. Detailed family history and clinical examination was obtained. A screen for Wilson's Disease was performed. This included a slit lamp examination, 24 hour urine copper, serum ceruloplasmin and copper estimations. The peripheral blood film was examined to exclude acanthocytosis. All patients had CT scan or MRI of the brain to exclude structural lesions. Blood was taken for DNA isolation after informed consent and sent to the Department of Molecular Medicine, Karolinska Hospital, Stockholm, Sweden for DNA studies.

Genomic DNA was isolated from peripheral blood lymphocytes using standard method. Polymerase chain reaction (PCR) was performed to amplify the (CAG)_n repeats in the HD and dentatorubral pallidolusian atrophy

(DPRLA) genes as previously described.^{2,3} For HD, the primers HD3 (5' CCTTCGAGTCCCTCAAGTCCTTC 3') and HD482 (5'GGCTGAGGAAGCTGAGGAG 3') were used. Primer HD482 was end labelled with ³²P-dATP using T4 polynucleotide kinase. PCR reactions were performed in a total volume of 25ul containing 50-100 ng of genomic DNA, 2M MgCl, 50 mM Kcl, 20mM Tris pH 8.4 , 3.5% formamide, 15% glycerol , 200 uM of each dNTP, 0.5uM of each primer and 1.25U of *Taq* DNA polymerase. Thermal cycling conditions were 96 C for 3 min , 35 cycles for 1 minute , 60 C for 1 min , with final extension at 72 C for 7 min. The PCR products were resolved on a 6% denaturing polyacrlamide gel, dried and subjected to autoradiography overnight. For DPRLA primers B-371 (5' CACCAGTCTCAACACATC 3') and B-371 (5'CCTCCAGTGGGTGGGGAAATGCTC 3') were used. The reaction and cycling conditions were the same as HD.

The DNA samples were also tested for dentatorubral and pallidolusian atrophy (DPRLA) as this illness can mimic the clinical features of HD. The causal mechanism for

DPRLA is related to an expanded (CAG)_n repeat within the DPRLA gene on chromosome 12p^{4,5}.

RESULTS

There were seven unrelated patients that had expanded CAG repeats on DNA testing with 14 other symptomatic relatives. The normal range of CAG repeats in controls were up to 35 CAG repeats. An excess number of repeats was taken as a positive test and diagnosed as a definitive case of HD. In a large worldwide study of the HD mutation, the cut off of 35 repeats for normals has a sensitivity of 98.8 percent (95 percent confidence interval) and a specificity of 100% (95 percent confidence interval)³.

The demographics, age of onset, family history*, number of CAG repeats, origins and the presence of inter-racial marriages are tabulated in Table 1. Patient 1 was previously reported as a clinical probable case of HD.⁶ Figure 1 shows the autoradiograph of the DNA analysis of patient 1 tested positive for HD compared to normal controls and a known case of HD. The clinical features of the patients are tabulated in Table 2. As shown, choreathetosis and cognitive decline was present in all patients.

TABLE 1: The demographics, family history, CAG repeats and ancestral origins of the patients.

Patient	Race	Age	Age of Onset	Sex	Number of CAG repeats	Family History	Affected relatives	Ancestral origin	Inter-racial marriage with Caucasian
1	Malay	43	33	male	40	yes	Maternal grandmother, mother and two maternal aunts	Malaysia	No
2	Indian	36	24	female	50	yes	Brother	Tamil Nadu, India	No
3	Chinese	46	40	female	40	uncertain	None	Guangdong, China	No
4	Indian	49	47	male	42	yes	Mother	Punjab, India	No
5	Chinese	52	45	male	45	yes	Mother and two sisters	China, Ireland?	Yes
6	Chinese	49	46	female	43	yes	Mother and maternal uncle	Guangdong, China	No
7	Chinese	50	47	male	41	yes	Father	Fujian, China	No

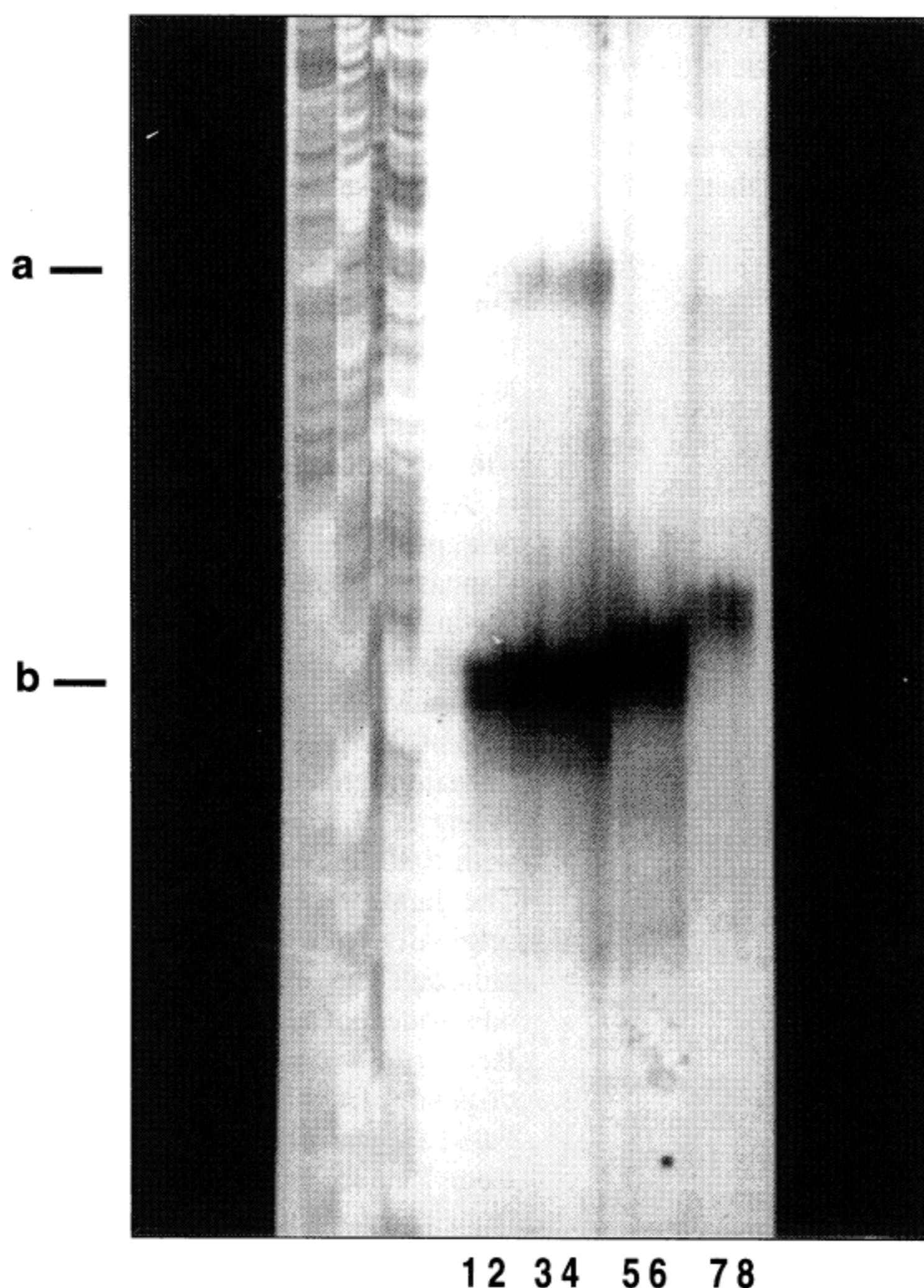


FIG. 1: The first column from the left show marker for the size of repeats. Columns 1, 2 are from patient 1 with HD. It showed higher bands (marker a) of about 40 repeats and the lower and darker bands (marker b) are the normal alleles with about 19 repeats. Columns 3,4 is the analysis of the same patient's DNA confirming similar findings. The subsequent samples 5,6 are normal controls and lastly 7,8 are from an affected individual with 39 repeats.

TABLE 2: Clinical features of the patients

Patient	Chorea athetosis	Tremor	Rigidity	Dystonia	Ataxia	Cognitive decline	Change of affect
1	+++	-	-	-	-	++	+
2	+++	-	-	-	-	+	-
3	+++	-	-	-	-	+++	++
4	++	-	-	-	-	+	-
5	++	-	-	-	-	++	+
6	++	-	-	-	-	++	+
7	++	-	-	-	-	++	++

+++ severe

++ moderate

+ mild

Change in affect was noted in 6/10 (75%) of patients. Three of the asymptomatic offsprings in patient 5 were tested positive for the HD gene. The length of the CAG repeats were 50, 46 and 70 respectively, longer than that of their father (45).

DISCUSSION

The discovery of a novel gene containing a trinucleotide repeat (CAG)_n on chromosome 4p16.3¹ has enabled cases of HD that were clinically diagnosed to be confirmed and also allows predictive testing of asymptomatic relatives of HD patients. The nationwide HD registry that was set up at the University of Malaya Medical Centre, Kuala Lumpur showed seven confirmed patients with excessive trinucleotide repeats.

The prevalence of HD in the west is estimated at 5 -10 persons per 100,000 population.⁷ However, in Asia and elsewhere the prevalence of HD is approximately one tenth of the Western population. The prevalence is 0.4 per 100,000 population in Hong Kong⁸, 0.65 per 100,000 in Japan⁹ and 0.5-1.0 per 100,000 in Zimbabwe, Africa.¹⁰ In India, there are case reports from Punjab and northern India.^{11,12,13} The age adjusted prevalence of HD among Indian subcontinent immigrants in Britain is 1.75 per 100,000.¹⁴ Until 1990, there were 69 case reports of HD in China⁸, however no accurate prevalence data is available. Elsewhere, rare case reports of HD are reported in Sudan¹⁵ and Saudi Arabia.¹⁶

In South East Asia the epidemiology data for HD consists mainly of case reports. In Singapore, HD has been reported in a Chinese family¹⁷ and lately in a second generation family Indian family from south India.¹⁸ One presumptive case was reported in an indigenous Malay family in Malaysia.⁶ This patient was traced in this study and blood was taken for DNA analysis. He is reported as patient 1 in this series. There are case reports of HD in Thailand.¹⁹ No accurate data could be obtained from Indonesia and Philippines.

The possible explanation for the wide variation in the prevalence of HD in different communities is unsatisfactory. There are advocates of a European origin for the mutation resulting from migration with inter-racial mixture of blood causing lower prevalence in non-European countries.⁸

There were four Chinese patients, two Indians (one Punjab, one Tamil) and one Malay in this study. The sex composition was four males and

three females. The average age was 39.2 years old with the average age of onset being 33.5 years old. A positive family history was seen in six out of seven patients (85.7%). The family history is suggestive of autosomal dominance inheritance in most of the cases. The family history of patient 3 was uncertain as all related family members have died. The number of trinucleotide repeats ranged from 40 - 50. The age of onset at the fourth and fifth decades is consistent with the low number of abnormal trinucleotide repeats.²⁰

As far as we can determine, there was only one probable inter-racial marriages with a Caucasian in the ancestry of the patients. Patient 5's maternal grandfather was said to be a Caucasian of Irish descent. Patient 1 is the first ethnic Malay to be confirmed to have HD. Patient 2's parents were Indian Muslims who have immigrated from Tamil Nadu, South India. Wong et al from Singapore reported a family with 5 siblings with clinical features of HD.¹⁸ The family was said to be ethnically Indian originally from a small village in South India, although the exact ancestral origin was not mentioned. One of the Caribbean families of Beaubrun's report of HD cases in Canada originated from Madras, capital of Tamil Nadu.²¹ Patient 4 was descendant of immigrant parents from Punjab, India. All reports of HD cases from the Indian subcontinent were from the northern states and Pakistan, the reported cases of HD among the Indian subcontinent immigrants in UK also originated from Punjab and northern India.¹⁴ The north and south Indians are linguistically and genetically different. Most of the Indians in Malaysia are descendants of immigrants from the southern states of India, with only about 10% from Punjab in the North.

As 73 % of the cases of HD in China were along the coastal province, Leung et al proposed the migration hypothesis of European ancestry for the Chinese HD patients. The suggestion was that after the Nanking Treaty in 1842, with the secession of Hong Kong to the British and the opening of five coastal cities; namely Shanghai, Ningbo, Fuzhou, Xiamen and Guangzhou, there was racial admixture of Chinese and White populations, and with it the arrival of HD to China. The "ultra-stable" structure of the Chinese culture, hostility of the inland population towards Westerners helped limit the extensive spread of the disease.⁸

There are several social and cultural reasons that this hypothesis is likely to be false. The ethnic Chinese in Malaysia are mostly second

generation immigrants from China whose parents migrated to Malaysia during the early part of this century due to poverty and civil war in China. Genealogy of Chinese families are well kept due the keen sense of ancestry among the Chinese. Hence, the family ancestry history obtained from our Chinese patients is reliable. There is only one possible Caucasian ancestor among the four Chinese families. There are no phenotypic features to suggest Caucasian ancestry among our patients or relatives after only four or at most five generations from the opening of the port cities during the Nanking Treaties.

Although the Chinese patients with HD in our series originated from the Southern coastal Provinces (Fujian and Guangdong) which had cities opened to the west (Fuzhou, Xiamen, and Guangzhou), their ancestors were from small villages such as Nanan, Yongchun in southern Fujian (patient 7) and Puning in Northern Guangdong (patient 6) rather than the port cities mentioned above. In the early part of this century, these small villages had poor access to the port cities. Travelling to the nearest port, such as from Puning or Yongchun to Xiamen involved several days of walking.

In addition, only men travelled alone to South East Asia. Women did not travel unless accompanying their husbands. Traditional Chinese culture also forbade Chinese women to have close contact with foreigners. Prostitution as a profession for women was deeply despised and extramarital sexual contact was strongly forbidden. Hence, it was highly unlikely that women ancestors of these patients would have sexual contact with asymptomatic Caucasian HD men resulting in the said admixture of genes.

There is also mounting evidence against the migration hypothesis from the current genetic literature. Although the nature of the abnormal trinucleotide repeats of the HD gene in Chinese is similar to that reported in the Caucasian HD patients, a majority of our patients have a small (<50) number of excessive CAG repeats.²² Among the Chinese in Taiwan, the number of trinucleotide repeats range from 40 to 58; 65.7% of the alleles had 40 to 45 CAG repeats and only 17.1% consisted of more than 50 repeats.²³ The number of CAG repeats from mainland China for seven affected HD patients range from 44 to 53.²⁴ The number of CAG repeats in patients from the West range from 37 to 86²⁵, 16 to 70²⁶ and 36 to 121³ in three different Western studies. Hence, the size of abnormal CAG repeats in HD

disease in the West differ from areas of low prevalence.

Secondly, it has been found that the differences in the CAG trinucleotide polymorphism at residue 2642 is significantly correlated with CAG size on normal chromosome. However, this glutamic acid polymorphism is absent in chromosomes of Japanese, African Blacks and Chinese.²⁷

Thirdly, the CCG triplet sequence immediately 3' adjacent to the CAG repeat is polymorphic. The two main triplet repeat sequence are alleles of seven or ten repeats.²⁸ The Japanese HD chromosomes show strong linkage disequilibrium with the (CCG) 10 but the Western HD chromosomes are associated with (CCG)7.²⁹⁻³¹

There is a highly significant inverse relationship between CAG and CCG repeat lengths in normal chromosomes.²² In populations with lower prevalence of HD such as Japan, China and African Blacks the CAG repeats in normal chromosomes are fewer and the distribution of the CCG repeats are different from the West.²² Hence, this further suggests that the origin of the HD gene is from different gene pools.

These reasons provide evidence that sporadic cases of HD arise from new mutations from parental intermediate allele which are unstable in meiotic transmission in areas of low prevalence of HD³². The development of new mutations is also the most likely the explanation for the HD cases seen in this region.

Normal Western Europeans have mean CAG repeats size of 19.3 but in races that have a lower prevalence, the number of CAG repeats are lower; for example Blacks 16.9, Chinese 16.6 and Japanese 16.7.²² The larger mean CAG repeat size among the Western Europeans may explain the predisposition to mutation and higher prevalence of HD in the population.

Genetic counselling was given to asymptomatic offspring of patient 5. Three of the offspring tested positive for the HD gene. The length of the CAG repeats were longer than their father. The phenomenon of anticipation in successive generations of HD patients is explained by increasing repeat lengths of unstable trinucleotide repeats.

The number of CAG repeats among our patients were small (< 50). Large CAG repeats (> 50) usually have paternal transmission²⁰ and early onset.^{20,33} A large number of trinucleotide repeat length is also associated with a higher rate of progression of clinical symptoms.³⁴

We screened four other patients with age range from 16-70 years with choreathetosis and varying degree of cognitive decline for HD. The mutation for HD and DPRLA were negative. One had an autosomal dominant family history. These patients with clinical pictures resembling HD may represent as yet an unknown genetic mutation.

In conclusion, the interim results of the HD registry has shown that HD exist in all major racial groups in Malaysia. All cases have in excess of 35 (CAG)_n trinucleotide repeats on chromosome 4p16.3. The migration theory of European genes causing racial admixture as the explanation for the low prevalence among our patients is not plausible. It is most likely due to separate mutation of the HD gene.

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