Molecular genetic analysis of cytosine-adenine-guanine repeats in huntingtin gene

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ABSTRACT

Background: Huntington's disease (HD) is a dominantly transmitted progressive neurodegenerative disorder due to abnormal expansion of cytosine-adenine-guanine (CAG) repeats in the huntingtin gene. **Materials and Methods**: The study involved a total of 35 HD patients identified through clinical evaluations. CAG repeats expansion analysis was conducted using polymerase chain reaction (PCR) to confirm positive cases. **Results:** Employing CAG flacking PCR in 35 cases, 19 tested positive, predominantly displaying CAG repeats in the range of 40–50. Noteworthy cases include a 5-month-old patient with 44 CAG repeats and an 80-year-old patient with 46 CAG repeats. **Conclusion:** The identification of the genetic defect in HD permits direct genetic testing to comprehend the complexities of HD in India, aiming for effective approaches in diagnosis, treatment, and prevention.

Key words: *Cytosine-adenine-guanine repeats, Huntingtin gene, Huntington disease, Indian population, Prevalence*

Huntington's disease (HD) is a neurodegenerative, progressive, autosomal dominantly inherited disorder characterized by motor disturbances, cognitive decline, and psychiatric manifestations. HD is associated with an expanded sequence of cytosine-adenine-guanine (CAG) repeats in the Huntingtin (HTT) gene located on chromosome #4p16.3 [1,2]. This expansion leads to the production of an abnormally long stretch of associated protein, resulting in a gain-of-function mutation in HD.

HD causes movement, cognitive, and psychiatric disturbances with a wide spectrum of signs and symptoms. There have been no detailed epidemiological studies from India. The overall prevalence of HD in Asia was 0.40/100,000 much compared to that of 5.70/100,000 in Europe, North America, and Australia [3,4]. Indian subcontinent represents one of the most ethnically and genetically diverse groups in the world. HD shows that signs of an imbalance of neurotransmitters in the basal ganglion have been considered a major culprit leading to the pathophysiology of movement disorders [5].

The reports on HD in various regions of India have been published, but determining the exact prevalence proves challenging. The individuals unaffected by HD typically possess 10–26 CAG repeats, while an intermediate range is defined as 27–35 repeats. CAG repeats falling 36–39 are considered incomplete or reduced penetrance, and individuals with 40 or more CAG repeats exhibit full penetrance, ultimately developing HD [6,7].

This study aims to find the gap by molecular basis of differences in the prevalence of HD among various populations, which is unclear. The primary objective is to investigate the prevalence of HD in India where approximately over 0.2 million people may be at risk [8].

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MATERIALS AND METHODS

The prospective study was conducted between 2023 and 2024 at the Molecular Genetic Unit of S. N. Gene Laboratory Private Ltd., Surat, India. The selection of patients was based on the clinical criteria for HD. The informed consent was taken from all the selected HD patients. Molecular genetic analysis for the expanded CAG repeat length was performed on 35 patients. The polymerase chain reaction (PCR) for amplification was done with one cycle of initial denaturation at 95% for 10 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 60°C o for 15 s, and final extension at 72°C for 5 min. CAG repeat length was determined after the amplification of genomic DNA. Electrophoresis of PCR amplified product was carried out on 2% agarose gel with appropriate negative control and DNA ladder (100bp) DNP bands were visualized using a gel documentation system.

RESULTS

A total of 35 clinically identified unrelated HD patients were analyzed for CAG repeat expansion at the HD locus (Table 1). Of 35 samples, HD mutations were found in 19 (54 %) patients, while 16 (46%) patients were classified as normal, among affected

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Table 1: Details of patients and number of CAG repeats detected in 35 samples following molecular analysis

No.	Age	Sex	Repeats	CAG repeats detected	Allele description
1.	9	Male	CAG	20	Normal
2.	37	Male	CAG	46	HD full penetrance
3.	26	Male	CAG	21	Normal
4.	36	Female	CAG	40	HD full penetrance
5.	60	Male	CAG	24	Normal
6.	26	Male	CAG	61	HD full penetrance
7.	30	Female	CAG	13	Normal
8.	35	Male	CAG	48	HD full penetrance
9.	23	Male	CAG	27	Normal
10.	30	Male	CAG	46	HD full penetrance
11.	35	Male	CAG	48	HD full penetrance
12.	23	Male	CAG	27	Normal
13.	36	Female	CAG	87	HD full penetrance
14.	23	Male	CAG	27	Normal
15.	30	Male	CAG	46	HD full penetrance
16.	41	Female	CAG	29	Normal
17.	25	Male	CAG	21	Normal
18.	54	Male	CAG	23	Normal
19.	62	Male	CAG	25	Normal
20.	49	Male	CAG	21	Normal
21.	48	Male	CAG	26	Normal
22.	36	Male	CAG	58	HD full penetrance
23.	27	Male	CAG	24	Normal
24.	47	Male	CAG	57	HD full penetrance
25.	25	Male	CAG	42	HD full penetrance
26.	43	Male	CAG	44	HD full penetrance
27.	36	Female	CAG	48	HD full penetrance
28.	63	Male	CAG	45	HD full penetrance
29.	47	Female	CAG	47	HD full penetrance
30.	19	Male	CAG	25	Normal
31.	80	Male	CAG	46	HD full penetrance
32.	5 Months	Male	CAG	44	HD full penetrance
33.	9	Male	CAG	25	Normal
34.	25	Male	CAG	42	HD full penetrance
35.	32	Female	CAG	48	HD full penetrance

CAG: Cytosine-adenine-guanine, HD: Huntington's disease

individuals, 14 (40%) were males and 5 (14%) were females. Age at onset of disease varied from 5 months to 80 years. The majority of the positive cases showed CAG repeats in the range of 40–50. The youngest patient was 5 months with 44 CAG repeats and the oldest was 80 years with 46 CAG repeats.

The study identified 19 positive cases with abnormal expansion of CAG repeats, confirming full penetrance, of which 14 (40%) were males and 5 (14%) were females, whereas 16 (46%) patients were found to be unaffected for CAG repeats repeat expansion consistent with HD and were thus classified as normal. The range of CAG repeats varied from 40 to 87 in HD-positive patients, and the age of onset spanned from 5 months to 80 years. The maximum number of cases was 15 (43%), with CAG repeats in the range of 40–50 (Table 1).

DISCUSSION

The findings of our study contribute significantly to the understanding of HD within the Indian population. Despite HD being a diagnosed condition for over 50 years, our investigation underscores the existing knowledge gaps among health-care professionals in India and the inadequacy of resources dedicated to HD awareness. The estimated conservative figure of 75,000 HD patients in India indicates a substantial risk to over a million individuals [9], emphasizing the urgency for comprehensive studies to inform health-care practices and resource allocation.

Our genetic analysis revealed abnormal expansion of CAG repeats with full penetrance in 19 positive cases. The successful amplification and confirmation of these cases through PCR highlight the reliability of our methodology. The range of CAG repeats observed in HD-positive patients (40–87) and the wide age of onset (5 months–80 years) demonstrate the heterogeneity of HD within the Indian population.

Comparative analysis with previous studies conducted in India and neighboring countries revealed consistency in the age of HD onset. For example, a study in Western India reported 361 cases with CAG repeats in the range of 37–51 [2], while an East Indian study conducted on 75 patients (male 57.3% and female 42.7%) with a mean age of 37.12 documented repeats with 40–79 [10]. Similarly, a study conducted in South India with 26 HD patients reported CAG repeats ranging from 39 to 85 [11]. Another study conducted from Karnataka, Banglore, reported 39–73 CAG repeats from 92 patients they have studied [12]. The study had some limitations; our is a diagnostic laboratory and most of the suspected cases are referred to us for genetic study. Some cases might have been missed. Socioeconomic and financial factors may also play a role in testing prices.

This alignment underscores the robustness of our results and their alignment with existing knowledge in the region. Contrary to global studies reporting reduced penetrance alleles (36–39 CAG repeats) in asymptomatic individuals, our study did not detect such alleles. The absence of reduced penetrance alleles in our cohort contrasts with estimates of HD prevalence in Western populations, where 5–10 individuals per 100,000 are affected. This discrepancy emphasizes the need for a comprehensive epidemiological study in India to ascertain the exact prevalence of HD. Molecular genetic modeling suggests that the prevalence of HD in India may be lower than in European populations, necessitating focused efforts to understand the unique genetic landscape of the Indian population [13].

While our study contributes valuable insights into the prevalence and characteristics of HD, it also highlights the complexity of diagnosing and understanding this neurodegenerative disorder. The identification of full penetrance cases suggests a potentially severe impact on affected individuals and their families, underlining the importance of early detection

and intervention strategies. Looking ahead, advancements in genetic diagnostics hold promise for identifying more conditions, but the translation of research into specific treatments will likely take time. Our study underscores the importance of ongoing research efforts and epidemiological studies to inform public health strategies and clinical practices for HD in India.

CONCLUSION

Identifying molecular genetic defects in CAG repeats within the Huntingtin gene allows us to ascertain the prevalence of Huntington's disease (HD) in India. Such knowledge is invaluable for enhancing both diagnosis and treatment strategies.

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