HEREDITARY PROGRESSIVE DYSTOMIA : AN INITIATION CODON MUTATION IN THE GTP CYCLOHYDROLASE I GENE

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Abstract: We studied the GTP cyclohydrolase I gene in a patient with hereditary progressive dystonia with marked diurnal fluctuation (HPD)/dopa-responsive dystonia (DRD). The patient was heterozygous for a G to C substitution in the initiation codon of this enzyme gene. As this transversion produced a new site for *Taq* I, the restriction site analysis ascertained that the mutation was specific for this patient. The putative translation, starting from the next AUG codon, will produce 46-amino acid peptide without GTP cyclohydrolase I activity. This is a new type of molecular deficit in the GTP cyclohydrolase gene, that causes HPD/DRD.

Index Terms

hereditary progressive dystonia, dopa-responsive dystonia, fluctuation

INTRODUCTION

Hereditary progressive dystonia with marked diurnal fluctuation (HPD)/dopa-responsive dyntonia (DRD) is a childhood-onset, postural dystonia that is alleviated by low doses of levodopa without adverse effects[1-4]. This disease transmits by autosomal dominant mode with variable penetrance. There is a marked female predominance. The GTP cyclohydrolase I gene has been currently identified as the causative gene for HPD/DRD[5]. The several genetic mutations and reduced enzyme activities have been reported in patients[5-9]. Here we show the first HPD/DRD case associated with an initiation codon mutation in the GTP cyclohydrolase I gene.

CASE STUDY

A 35-year-old Japanese woman had a history of gait difficulty due to intermittent internal rotation of her legs since the age of eight. She could walk in the morning, but became immobile toward the evening. Small doses of levodopa gave marked and sustained therapeutic response. Genomic DNAs were extracted from peripheral blood leukocytes of the patient and unrelated normal controls. All exons and splicing junctions of the GTP cyclohydrolase I gene were amplified using PCR on genomic DNA. Primer sequences and experimental conditions for PCR amplification and sequencing analysis were the same as those previously reported by Ichinose et al. and Hirano et al.[5-7]. The patient was heterozygous for a G-to-C substitution in the initiation codon (Figure 1a). As the mutation predicted the generation of *Taq* I restriction site, genomic fragments containing the mutation were examined by restriction fragment length polymorphim analysis method. The patient had extra fragments (50 and 436 bp) in addition to

normal fragments (16 and 486 bp), as snown in Figure 1b. These restriction patterns and the presence of two different allelic nucleotide sequences endorsed that the patient was heterozygous for the gene mutation. This base change was not a DNA polymorphism, because it was undetectable on 100 chromosomes from unrelated normal controls.

In higher eukaryotes, the first AUG triplet within an acceptable sequence context serves as the initiation codon. The sequence of GCCGCC (A/G) CCAUGG is believed to be optimal context[10,11]. The A of the AUG codon is designated +1, with positive and negative integers proceeding 3' and 5', respectively. The G at position +4 and the purine at position −3 are specifically important in defining a good context. There are ten inframe AUGs and six outframe AUGs in the mRNA coding region for wild type of GTP cyclohydrolase I[12]. In our patient, the G-to-C substitution abolishes the first AUG codon. The next AUG codon, which occurs out of frame at the position corresponding to amino acid number 20, lies in a strong context ; the G at position +4 and the purine at the −3 position are both in favorable alignment (GGUGCAGCAAUGG). Therefore, this AUG is most likely to be recognized as the initiation codon, and a frame shift will occur and produce a UGA termination codon 139 nucleotides downstream. The putative translation will produce 46-amino acid peptide without GTP

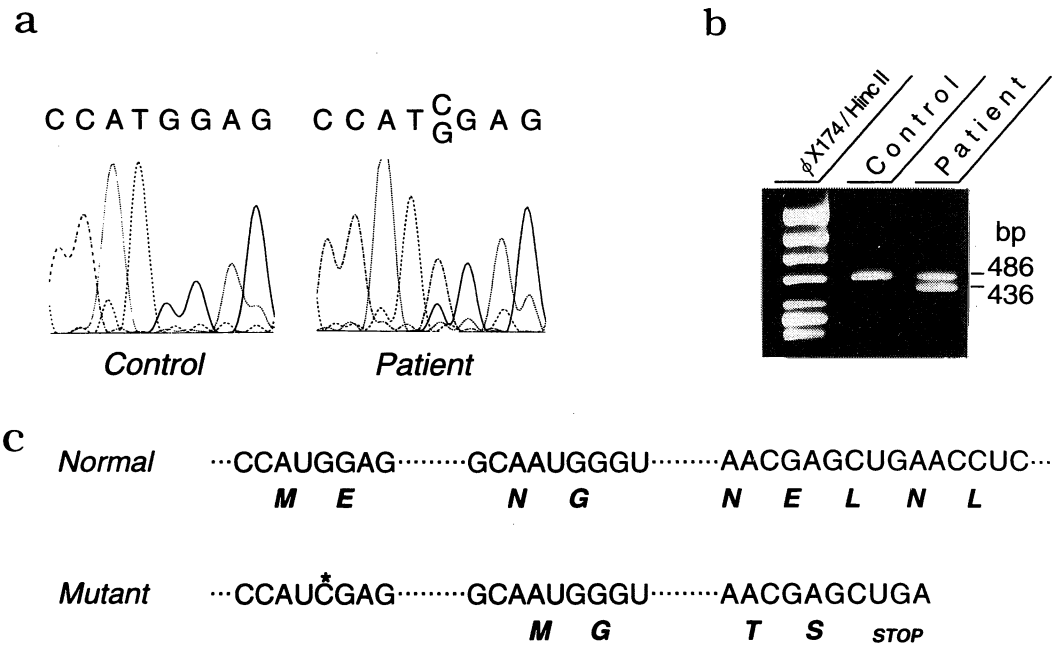

Fig. 1. (a) The control is homozygote of G at the third letter in the initiation codon ATG. The HPD patient is heterozygote of C and G at the same position.
(b) Identification of the initiation codon mutation by *Taq* I restriction anlysis. The patient shows two extra bands (50 and 436 bp) in addition to normal bands (16 and 486 bp). The two small bands (16 and 50 bp) are not visible.
(c) Upper mRNA sequence encodes normal GTP cyclohydrolase I, and lower sequence does mutant one. An asterisk shows the G-to-C mutation at the third letter of the initiation codon. When the next outframe ATG is used as an initiation codon, a resulting product will encode non-functional 46-amino acid peptide. The sequence is based on the published data of Togari et al.[12]).

cyclohydrolase I activity (Figure 1c). It is unlikely that the inframe AUG codon at the amino acid position 102 is used as an initiation codon. This methionine codon is flanked by G at position −3 but C at position +4, an unfavorable arrangement for translation initiation (GCCUCGGCCAUGC). Other AUGs should not be recognized as the initiation codon, because they are in the weakest contexts (not shown). Although some mRNA translation is initiated at non-AUG triplets including ACG and CUG, they may be only rarely recognized as the initiation signal[13-15]. In this study, fresh lymphocyte specimens from the patient were not available for enzyme activity measurement. On the basis of published data of Ichinose et al.[4], we could predict that the mutant GTP cyclohydrolase I activity was decreased. Ichinose et al. described two patients (Family Sa) associated with the frameshift mutation in the GTP cyclohydrolase I gene[7]. The two-base insertion mutation occurred just after the initiation codon (ATGGAG-to-ATGGGGAG), resulting in a frame shift after the starting methionine. The enzyme activities in phytohemagglutinin (PHA)-stimulated mononuclear cells from these patients were less than 20 % of nomal value. Mutations in an initiation codon are reported in a variety of hereditary diseases including β-thalassemia[16] and phenylketonuria[17]. These facts have tempted us to conclude that the initiation codon mutation impairs GTP cyclohydrolase I function, leading to clinical phenotypic expression.

CONCLUSION

We have reported a new type of molecular defect in the GTP cyclohydrolase I gene, which causes HPD/DRD. Further investigation of HPD/DRD genetic deficits may provide the molecular basis to clarify basal ganglia pathology of other diseases such as Parkinson's disease.

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