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ORIGINAL ARTICLE

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# Nonsense mutation in *TITF1* in a Portuguese family with benign hereditary chorea

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**Abstract** Benign hereditary chorea (BHC) is an autosomaldominant disorder of early onset characterized by a slowly progressing or nonprogressing chorea, without cognitive decline or other progressive neurologic dysfunction, but also by the existence of heterogeneity of the clinical presentation within and among families. The genetic cause of BHC is the presence of either point mutations or deletions in the thyroid transcription factor 1 gene (*TITF1*). We studied a Portuguese BHC family composed of two probands: a mother and her only son. The patients were identified in a neurology out-patient clinic showing mainly involuntary choreiform movements since childhood, myoclonic jerks, falls, and dysarthria. We performed magnetic resonance imaging (MRI), electroencephalogram (EEG), nerve conduction studies, thyroid ultrasound scan, biochemical thyroid tests, and electrocardiogram (ECG). We excluded Huntington disease by appropriate genetic testing and sequenced the entire *TITF1* gene for both patients. The patients showed MRI alterations: (1) in the mother, abnormal hyperintense pallida and cortical cerebral/cerebellar atrophy; and (2) in the son, small hyperintense foci in the cerebellum and subtle enlargement of the fourth ventricle. Sequence analysis of the *TITF1* gene in these patients revealed the presence of a heterozygous C > T substitution at nucleotide 745, leading to the replacement of a glutamine at position 249 for a premature stop codon. A previously undescribed nonsense mutation in the *TITF1* gene was identified as being the genetic cause of BHC in this family.

**Keywords** Choreoathetosis · Nkx2.1 · Thyroid dysfunction · Huntington disease · Pulmonary alterations

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## Introduction

Benign hereditary chorea (BHC), first described in 1967 [1], is a dominantly inherited disorder of early onset that is characterized by a very slowly progressing or nonprogressing chorea without cognitive decline or other progressive neurologic dysfunction. This is in contrast with the dementia and progressive course of chorea, which are the hallmarks of classical Huntington disease, another inherited movement disorder. Heterogeneity of clinical presentation exists within and among BHC families. Besides chorea, many of the published cases of BHC include atypical clinical features such as dystonia, myoclonic jerks, mild dysarthria or gait disturbances, and low-average intelligence [2].

Until recently, doubts existed concerning the definition of BHC as a separate and well-defined clinical entity [2]. However, the mapping and cloning of its causative gene on chromosome 14q [3], the gene encoding thyroid transcription factor 1 (TITF1), also known as *NKX2-1*, was even-

tually reported in 2002 [4]. TITF1 is a member of the NK2 family homeodomain-containing transcription factors [5, 6]. In adult human tissues, *TITF1* is exclusively expressed in the thyroid and in the lung [7]. However, during human development, the gene is expressed: (1) strongly in the ventral diencephalon (hypothalamic area and infundibulum) and in the telencephalon, more precisely, in the developing basal ganglia region (striatum and paleostriatum); (2) in the lung bud, firstly in the primary bronchi epithelia and at 9 weeks of gestation in the alveolar primordial epithelium; and (3) weakly in the thyroid primordium [8]. Mutations in this gene have been found in five independent BHC families of Dutch, North American, Welsh, Italian, and Canadian origin [4, 9–12], as well as in patients with a combination of choreoathetosis, hypothyroidism, and pulmonary symptoms of different origins [7, 13–18] (see Table 1 for review).

Moreover, evidence exists for the existence of genetic heterogeneity within BHC cases [9]. We have investigated the genetic basis of disease in a Portuguese family with BHC and identified a previously undescribed point mutation in the *TITF1* gene.

## Subjects and methods

*Family* Two patients (a mother and her only son, see Fig. 1a) were referred to the neurology out-patient's clinic because of "tremor." Clinical information was provided by both probands, who denied the existence of other affected family members beside themselves. Except for the proband's father, other relatives were unavailable for examination or genetic testing.

*Patient 1* H.P. is a 30-year-old male. Involuntary movements were first noted by his mother at the age of 10 months. He first walked at the age of 2 years and had

**Table 1** Mutations in the *TITF1* gene and their implications at the protein and phenotypic level

| Reference number | DNA sequence | Consequence at protein level | Protein domain localization | Mode of transmission | Phenotype | | |
|---|---|---|---|---|---|---|---|
| | | | | | Thyroid dysfunction | Pulmonary alterations | Choreoathetosis |
| 17 | Deletion, including the entire *TITF1* gene and the *PAX9* gene | Absence of protein | E | AD | CH | Severe | Severe |
| 15 | Deletion of chromosome 14q12-13.3, including the *PAX9* gene | Absence of protein | E | de novo | CH | Severe | Severe |
| 4, 9 | G713T | W238L | H | AD | CH | NA | Severe |
| 4, 9 | C727A | R243S | H | AD | NA | NA | Severe (cerebellar alterations, dysarthria) |
| 4, 12 | ΔG908 | G303fsX77 | N | AD | NA | NA | Severe |
| 4, 11 | 1.2-Mb deletion, including the entire gene | Absence of protein | E | de novo | NA | NA | Severe |
| 13 | 255insG | G86fsX322 (G115fsX322) | TN-H | de novo | CH | Severe | Severe |
| 10 | −2A>T splice acceptor site of intron 2 | I155fsX2 | TN-H | AD | Absent | Absent | Severe |
| 7 | Deletion of chromosomal region 14q11.2-q13.3, including the entire gene | Absence of protein | E | NA | CH | Severe | Severe |
| 7 | G2626T | V235F | H | de novo | CH | Severe | Severe |
| 7 | 2595insGG | L224fsX3 | H | de novo | Mild | Mild | Severe |
| 7 | C2519A | S199X | H | NA | CH | Absent | Severe |
| 7 | C1302A | C117X | TN-H | NA | CH | Severe | Muscular hypotonia |
| 16 | −2A>G splice acceptor site of intron 2 | I155fsX2 | TN-H | AD | CH | Severe | Severe |
| 14 | 859–860insC | −Q287fsX121 (−Q317fsX121) | C | de novo | CH | Severe | Severe |
| 18 | G523T | E175X (E205X) | H | AD | CH | Severe | Severe |
| Current study | C745T | Q249X | H | AD | Absent | Absent | Mild/severe |

*E* Entire protein, *AD* autosomal dominant, *CH* congenital hypothyroidism, *H* homeodomain, *NA* information not available, *N* NK2-specific domain, *TN-H* sequence between the TN and the homeodomain, *C* carboxyl terminal (after the NK2-specific domain)

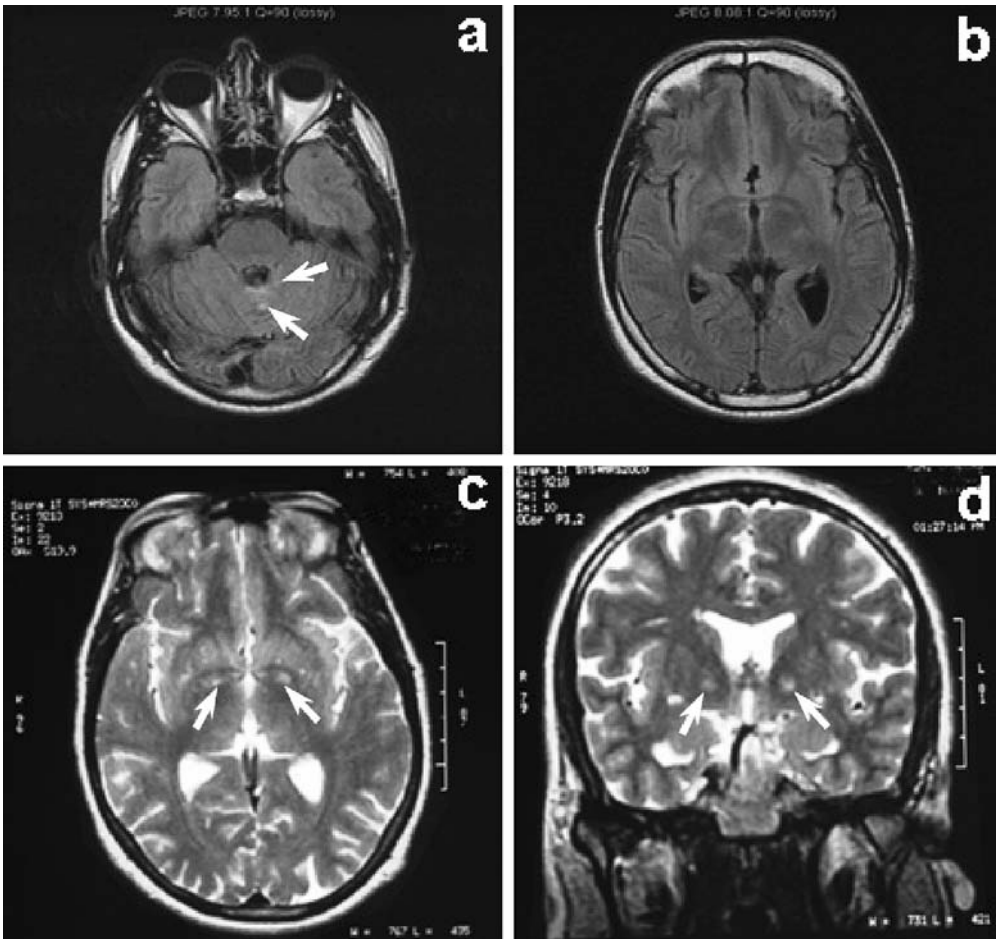


**Fig. 1** Brain magnetic resonance imaging of patient 1 (**a**, **b**) and patient 2 (**c**, **d**). Axial-T2-weighted images of patient 1 show multiple small hyperintense foci (*arrows*) in the superior vermis, both within the cortex and in the subependymal region (**a**), and no abnormalities in the basal ganglia (**b**). Axial (**c**) and coronal (**d**) T2-weighted images of patient 2 show bilateral and symmetrical foci of hypersignal in the basal ganglia involving the globus pallidus (*arrows*) as well as moderate cerebral cortical atrophy

frequent falls throughout childhood, which ceased around puberty. He said his first words at 18 months and has been a stutterer since then. Learning difficulties were noticed, and the patient was unable to complete high school. To the best of our knowledge, these learning difficulties were not assessed by cognitive measures at the time, although the patient was referred to a psychologist and followed for over a year after failing his fourth year at school twice (last year of primary school). He then went on to complete another four years at school, after which he quit and took an electrician's course by correspondence. There was no history of respiratory insufficiency. Congenital strabismus had been surgically corrected in childhood. The patient worked as an air-conditioning technician; his jerks did not interfere significantly with his professional performance.

The general examination of this patient was unremarkable (height 176 cm, weight 72 kg). He had a stuttering speech, which appeared explosive at times. Cranial nerve examination was normal, except for a left upper lid ptosis (postsurgical), without diplopia. There was mild spasticity of the lower limbs, bilateral pes cavus, and a bilateral hammer deformity of the first toe. He had quite subtle involuntary movements, which were choreiform in upper limbs and trunk. There was no tremor. The remainder of the neurological examination was normal.

*Patient 2* G.P. is a 59-year-old female. Information on the mother's past medical history was scarce. She walked at the age of 2 years. She remembered having had "ticks" all her life and insisted that they had been much worse than at present. She occasionally lost her balance and had a few major falls, one of which caused a luxation of her left elbow. There were no respiratory complaints. She had attended school only for 2 years and remembered having problems with learning. The patient was of low stature and obese (height 145 cm, weight 73.4 kg). She displayed inappropriate laughter, and her speech was sometimes of an explosive nature. Deep tendon reflexes were abolished. There was a symmetrical hypesthesia for pain distally in the lower limbs. Choreiform movements were striking and affected the whole body. In spite of her occasional loss of balance, she did not display a wide-based gait. There was no tremor or any other neurological abnormalities.

*Proband's father* The neurologic examination was normal except for the presence of bilateral pes cavus and bilateral hammer of the first toe.

*Additional investigations* We performed brain magnetic resonance imaging (MRI) studies of both patients as well as electroencephalogram (EEG), nerve conduction studies, a

thyroid ultrasound scan, biochemical thyroid function tests [free T3, T4, and thyroid-stimulating hormone (TSH)], and electrocardiogram (ECG). Huntington disease was excluded by appropriate genetic testing [19].

*Control population* We have extracted DNA from 1,000 anonymous Guthrie cards obtained from the national neonatal phenylketonuria (PKU) screening program using the Chelex method. The samples were randomly selected and

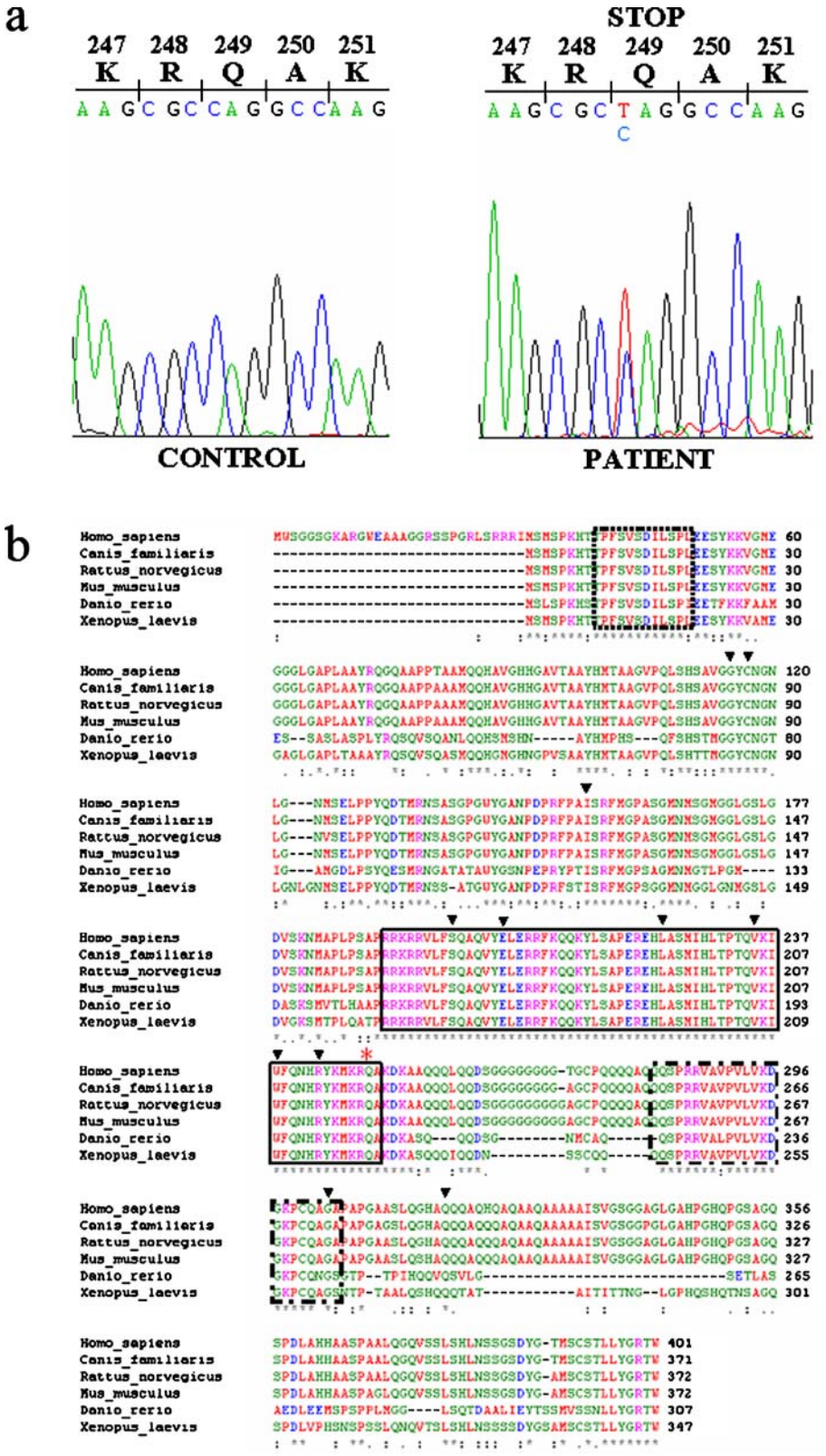


**Fig. 2** The $C^{745}>T^{745}$ mutation in the *TITF1* gene results in a substitution at codon 249 of a glutamine for a stop codon (*Q249X*). **a** DNA (sequencing chromatogram) and amino acid sequences of a normal individual (*left*) and of patient 1 (*right*). **b** Sequence alignment of human TITF1 with homologs of others species using Clustal W (*asterisk* indicates identical or conserved residues in all sequences, *point* indicates semi-conserved substitutions, and *colon* indicates conserved substitutions. The TN-, homeodomain and NK2-specific domains are represented as *boxes* relined with *dots*, *solid lines*, and *dashes*, respectively. The *red asterisk* indicates the nonsense mutation present in the Portuguese BHC family, and the *black arrows* show all the point missense, nonsense, and frameshift mutations reported in this protein. GenBank accession numbers of protein sequence are listed in "Subjects and methods"

included two individuals from each district of Portugal ($n$=100).

*Genotype analysis* After informed consent was obtained, genomic DNA was extracted from blood samples according to standard protocols using the Puregene system (Gentra). HD testing was performed, as described elsewhere by the use of polymerase chain reaction (PCR) analysis, using primers that flank the CAG repeat [19]. For mutation analysis of the *TITF1* gene, genomic DNA was amplified by PCR using primers flanking the exons of the gene as described [4]. Sequence analysis was performed by cycle sequencing using the same primers, and detection was performed in an ABI sequencer by a company (MWG Biotech).

*Bioinformatic analysis* All protein sequences were taken from the TrEMBL database. The accession numbers of human, dog, rat, mouse, zebrafish, and *Xenopus laevis* TITF1 sequences are AAP88775, P43698, XP_216720, P50220, NP_571664, and AAG17405, respectively. Multiple sequence alignment of TITF1 proteins was performed using the Clustal W program [20].

## Results

The brain MRI scan of the first patient, H.P., was unremarkable, except for subtle abnormalities of the cerebellum, which were of undetermined significance: in the vermis, both within the cortex and the subependymal region, multiple small hyperintense foci were seen (Fig. 1a). There was no contrast enhancement of these lesions after gadolinium injection. There was a small but definite enlargement of the fourth ventricle. The mother, G.P., displayed symmetrical foci of hypersignal in T2 weighted images, involving both globi pallida, as well as a moderate cerebral cortical atrophy (Fig. 1c,d). There was also cortical and subcortical cerebellar atrophy.

In both patients, EEG, nerve conduction studies, thyroid function, and lung function tests were normal. A thyroid ultrasound scan was also considered normal in both cases.

Regarding cognitive evaluation, patient 1 had a mini mental examination of Folstein (MMSE) of 30/30 and a low-normal intelligence (total WAIS−R=87). There was cognitive impairment of patient 2 (MMSE 16/22, WAIS−R=62, mentally retarded range).

No mutation was found in the *HD* gene, thus ruling out Huntington disease. In both cases, $(CAG)_n$ sizes were 17 and 27 triplets corresponding to a normal allele and to a normal class 2 allele of large size, respectively.

We have identified a heterozygous C > T substitution at position 745 of the *TITF1* gene in patients 1 and 2 (Fig. 2a). This nucleotide replacement was not present in 100 controls of Portuguese origin. The C745T mutation affects the two known splice variants of this gene [21, 22], and originates the substitution of a conserved glutamine at position 249 of the protein by a premature stop codon. The Q249X mutation is located at the very end of the helix III of the homeodomain and gives rise to a protein lacking its 153 C-terminal amino acids, including the entire NK2-specific domain (Fig. 2b).

## Discussion

We have identified a new family with BHC of Portuguese origin and provide additional confirmation that mutation of *TITF1* is a cause of this disorder in different populations. The clinical presentation of the patients in this family overlaps with that of the reported cases in the literature. The variability of the neurological signs found in one or both of our patients (e.g., spasticity, dysarthria, mental retardation, and falls) is in accordance with the heterogeneity of the published cases of BHC [2, 9]. Some of these features, such as pes cavus and hammer toes, seen in patient 1, were shared by the patient's father, and are therefore most likely not due to the *TITF1* mutation.

Magnetic resonance imaging descriptions of patients bearing *TITF1* mutations are scant. In the published BHC families with *TITF1* mutations, MRI abnormalities were either absent [4, 10], or unreported. In one of the more severe cases of nonprogressive choreoathetosis, congenital hypothyroidism and neonatal respiratory distress, associated with *TITF1* mutations, small, malformed pallida were described, as well as cystic masses in the posterior part of the sella turcica [7]. The observation of abnormal hyperintense pallida in patient 2 (the proband's mother) is in agreement with the fact that *TITF1* is expressed in the developing basal ganglia [8], and is consistent with the malformed pallida reported by Krude et al. [7], as well as with the absence of pallidal structures and basal forebrain TrkA-positive neurons in *Nkx2.1* knockout mice [23]. Since *TITF1* is only expressed during human development in the ventral forebrain [8], and no protein was detected in adult human brain [7], TITF1 is probably important to regulate the formation of basal ganglia, but not for their function, once properly developed.

The cerebellar abnormalities found in the MRI scan of patient 1, in the present report, have not, to our knowledge, been described before, and their significance is unclear. The Dutch BHC family, carrying a R243S mutation in TITF1 [4], presented cerebellar and pyramidal signs, but the patients' MRIs revealed no alterations [3]. The cortical cerebral and cortical/subcortical cerebellar atrophy seen in patient 2 can possibly correlate with the observation that *Nkx2.1* knockout mice have reduced numbers of cortical migrating cells expressing gamma-aminobutyric acid (GABA), DLX2, and calbindin produced in the pallidum [23].

The mutation identified in this family was a nonsense mutation at position 249 of TITF1, which was not present in a sample of the general population, including 200 chromosomes. The description of yet a novel mutation in *TITF1*, together with the fact that approximately 46% of the mutations reported in this gene were de novo mutations [4, 7, 13–15], suggests that BHC results from multiple mutational events worldwide.

The mechanism through which this nonsense mutation may be leading to a dominant disease is unknown. Loss

of function due to protein deletion or truncation, or to mRNA nonsense-mediated decay, may be occurring, leading to haploinsufficiency, as has been proposed [7, 10, 13]. Alternatively, dominant-negative disruption of the TITF1-containing transcriptional complexes may be the cause of disease.

We propose that simple haploinsufficiency does not fully explain the mechanism of disease in the spectrum of disorders associated to TITF1 mutations, including BHC. The phenotypic variability associated with *TITF1* mutations (Table 1) can be explained by the type, size, and/or localization of the mutation, and consequently, by the affected domain(s) of the protein in each case. Alterations of the protein producing a gain or loss of a specific function, associated to a certain domain, can be important for a determined tissue or in a specific stage of development, but not another.

The Q249X mutation gives rise to a protein containing both the TH domain and the homeodomain, that only lacks its last two amino acids of helix III, which are apparently not fundamental for its normal function, at least in the rat TITF1 and *Drosophila melanogaster* NK-2 homeodomain homologs [24, 25]. Nevertheless, the mutant protein lacks the 153 C-terminal amino acids, including the entire NK2-specific domain. This domain serves as a protein–protein interaction interface and is able to mask the transcriptional activation of both the N-terminal and C-terminal activation domains [26–28]; thus, it is probably important to regulate the activation of specific genes by TITF1 during development. Taking into account the phenotype of the two patients in this family, we propose that this mutant protein (if translated) is able to bind to its target DNA-binding sites and activate their transcription in the lung and thyroid (through a functional homeodomain) but fails to activate specific genes that are important for the embryonic development of the basal ganglia (the only stage in which TITF1 is expressed in the central nervous system) because it lacks the C-terminal domain.

However, at this time, there is not enough data available to support the aforementioned hypothesis since many of the reported cases have not been fully examined for the whole phenotypic spectrum of BHC. Additionally, since similar mutations, or even the same mutation, in *TITF1* cause different phenotypes (corresponding to several combinations of choreoathetosis, hypothyroidism, and respiratory alterations), other factors such as environmental exposure, hormonal and/or modifier genes can be contributing to this clinical variability.

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