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J. Neurol. Neurosurg. Psychiatry 2008;79;183-186; originally published online 26 Sep 2007; doi:10.1136/jnnp.2007.128413

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Frequency of GCH1 deletions in Dopa-responsive dystonia

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ABSTRACT

We performed a systematic study on the frequency of point mutations and deletions of the gene GCH1 in dopa-responsive dystonia (DRD). A total of 136 dystonia patients were studied. Fifty of these had a sustained response to oral L-Dopa therapy (group 1: definite diagnosis of DRD), whereas the response to L-Dopa was incomplete or not tested in 86 patients (group 2: possible diagnosis of DRD). We found a GCH1 point mutation in 27 patients of group 1 (54%) and in four patients of group 2 (5%). Of these, nine single and one double mutation have not been described before. GCH1 deletions were detected in four patients of group 1 (8%) and in one patient of group 2 (1%). Among GCH1 point-mutation-negative patients with a definite diagnosis of DRD (group 1), the frequency of GCH1 deletions was 17% (4/23). We conclude that GCH1 deletion analysis should be incorporated into the routine molecular diagnosis of all patients with DRD with a sustained response to L-Dopa.

Dopa-responsive dystonia (DRD), which was first described by Segawa et al.,1 is a progressive primary dystonia that is characterised by onset during childhood, circadian fluctuation of symptoms and a remarkable therapeutic response to L-Dopa.2–4 The clinical picture is frequently less typical and can vary remarkably among affected individuals. Presenting signs and symptoms can be focal dystonia, orthopaedic anomalies (eg pes equino-varus), adult-onset parkinsonism or psychiatric disturbances. Prevalence of DRD was given as 0.5 per 1 million,2 but may be significantly higher owing to underdiagnosis. Females are affected 2–3 times more frequently than males. The disorder is mostly inherited as an autosomal-dominant trait with reduced penetrance.

Dopa-responsive dystonia is frequently caused by mutations in the gene GCH1 that encodes GTP-cyclohydrolase 1 (GTPCH1). GTPCH1 is the rate-limiting enzyme in the synthesis of tetrahydrobiopterine (BH4), an essential cofactor of phenylalanine-, tyrosine- and tryptophan-hydroxylase. Therefore, depletion of BH4 as a consequence of mutations in GCH1 results in insufficient synthesis of tyrosine, dopamine and serotonin. There are also rare cases of autosomal-recessive DRD that can be caused by mutations in the tyrosine hydroxylase gene (TH).5 In addition, there is one report of autosomal-dominant DRD that is caused by a mutation in the sepiapterine reductase gene (SPR).6

Most mutations described in GCH1 are single base changes. Routine molecular diagnosis of DRD is usually restricted to the sequencing of the six exons of GCH1. As mutations mostly occur in the heterozygous state, deletions are not detected by sequencing. Deletion detection requires special methods such as quantitative real-time PCR (qPCR) or multiple ligation-dependent probe amplification (MLPA). To date, only few GCH1 deletions have been reported in DRD.7–10

We tested a large cohort of patients with features of DRD for point mutations and deletions in GCH1 in order to establish the frequency of GCH1 gene deletions and to define inclusion criteria for GCH1 deletion analysis in DRD.

PATIENTS

A total of 136 patients with dystonia referred for molecular diagnosis of GCH1 mutations. The patients were of German descent. Initially, the family history of these index cases was not known. Once a mutation was found in GCH1, however, molecular analysis was offered to additional family members. EDTA blood samples from these patients had been sent to our lab during 1997–2006. The patients were divided into two groups according to clinical criteria and response to L-Dopa. Group 1 included 50 dystonia patients with typical DRD symptoms (eg childhood onset of dystonia, circadian fluctuation) and a dramatic and sustained therapeutic response to L-Dopa without subsequent on–off phenomena (clinically definite DRD). Of these, 37 (74%) were female and 13 (26%) were male. The average age of onset was 7.9 years (range 0–23 years, information available in 52 patients). Daily L-Dopa dosages ranged from 20 to 600 mg. Group 2 included those dystonia patients in whom clinical data were incomplete and the L-Dopa response was not striking or not tested. In this group, 50 (58%) were female and 56 (42%) were male. The average age was 13.6 years (range 0–44 years, information available in 14 patients).

METHODS

DNA extraction and sequencing

DNA was extracted from peripheral blood lymphocytes of patients and controls according to standard procedures. All six GCH1 exons, as well as exon–intron boundaries, were sequenced with previously described primers.11

GCH1 deletion analysis

GCH1 deletion analysis was performed by qPCR in those patients in whom no point mutation had been detected in GCH1. The procedure of qPCR and evaluation of data were exactly as described previously.19 Of the GCH1 deletion-positive patients, additional family members (particularly...
first-degree relatives) were also analysed for GCH1 gene deletions.

**Paternity testing by microsatellite analyses**

Paternity testing was performed in a family with an apparently *de novo* mutation of GCH1. A total of 28 autosomal and 5 X-chromosomal short tandem repeat loci were analysed.

**RESULTS**

The patients were divided into two groups based on reported clinical features and their response to L-Dopa. A dramatic and sustained response to L-Dopa is the key feature for definite diagnosis of DRD and was reported in 50 dystonia patients (group 1). Group 2 included 86 cases with dystonia in whom DRD was suspected, but response to L-Dopa was not striking (reported doses were low) or was not tested before molecular testing.

Thirty-two point mutations in GCH1 were detected in 31 of the 136 patients (22.8%). The point mutations comprised 15 missense, two nonsense, one frame-shift and 14 splice-site mutations (fig 1). A double mutation (missense mutation Pro23Leu and nonsense mutation Gln110Stop) was found in one patient. One of these mutations (Pro23Leu) was derived from the father and the other (Gln110Stop) must have occurred *de novo*. The patient did not differ clinically from other patients with DRD. With the exception of one homozygous GCH1 missense mutation (Asp134Asn) in a patient with consanguineous parents, all other 31 GCH1 mutations were heterozygous. Ten of these, including the double mutation, are reported here for the first time (fig 1). Point mutations had occurred in 27 patients assigned to group 1 (27/50 = 54%) and in 4 patients of group 2 (4/86 = 5%). None of these sequence changes were found in 100 controls.

The 105 patients with no detectable point mutation were further tested for GCH1 exon deletions by qPCR (fig 1). We
detected heterozygous deletions of \textit{GCH1} in five patients. Four of these patients had been assigned to group 1 and one to group 2. The entire \textit{GCH1} gene (exons 1 to 6) was deleted in four of the five patients. In one of these patients, \textit{GCH1} was partially deleted (exons 3 to 6) (family 5; fig 2). Relatives of three of the five patients with a deletion were available for genetic testing (families 1, 2 and 5; fig 2). In two cases, the \textit{GCH1} deletion was proven to be familial. One of these familial \textit{GCH1} deletions (exons 1 to 6) occurred in a large family with four affected females in three generations and an asymptomatic male carrier (family 1; fig 2). The second familial \textit{GCH1} deletion was partial. It comprised exons 3 to 6 and was detected in a woman with classical DRD and her asymptomatic mother (family 5; fig 2).

Furthermore, one \textit{GCH1} deletion (exons 1 to 6) was proven to have arisen \textit{de novo} (patient II.2 of family 2; fig 2). Both parents had two copies of \textit{GCH1} and paternity was confirmed by analysis of 28 autosomal and 5 X-chromosomal STR polymorphisms. The majority of deletions (4/5) were found in patients of group 1 with typical DRD symptoms and sustained response to oral L-Dopa administration (4/50 = 8%). The age of onset was during childhood in all patients with \textit{GCH1} point mutations and deletions.
Interestingly, the only patient of group 2 with a GCH1 deletion (family 4; fig 2) was an older man in whom responsiveness to L-Dopa was only tested briefly. After 200 mg of L-Dopa did not yield immediate striking therapeutic improvement, L-Dopa therapy was discontinued. Higher doses were not tried.

**DISCUSSION**

We performed an extensive molecular study of GCH1 in a large cohort of 136 patients with clinical signs and symptoms of DRD. The majority of point mutations were detected in patients of group 1 (54%) compared with only 5% in group 2. Mutation types included missense, nonsense, frame-shift and splice-site mutations. Of these, four missense, one nonsense and four splice-site mutations, as well as the combination of the previously described missense mutation Pro23Leu with the nonsense mutation Ghn110Stop, have not been previously reported. The relative frequency of 47% nonsense mutations is comparable to that described in the literature (55% nonsense mutations in the recent frequency of 47% missense mutations is comparable to that described in previous publications) have been described. Independent of the size of the deletion, all affected persons had classical DRD.

In conclusion, the findings obtained in this large group of patients underline the importance of rigorous L-Dopa testing in patients with suspected DRD. GCH1 sequence analysis should be performed in all patients with dystonia plus a positive therapeutic response to L-Dopa. If a point mutation is excluded, GCH1 deletion analysis should routinely be performed in this well-defined group of patients. Our study indicates a high detection rate of GCH1 mutations (point mutations and deletions) by this diagnostic strategy. However, the analysis of additional genes in GCH1-negative cases (TH, parkin, SPR) is currently not feasible in a routine setting.

**REFERENCES**