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B Zirn, D Steinberger, C Troidl, K Brockmann, M von der Hagen, C Feiner, L Henke and U Müller

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Frequency of *GCH1* deletions in Dopa-responsive dystonia

B Zirn,[1] D Steinberger,[1,2] C Troidl,[1] K Brockmann,[3] M von der Hagen,[4] C Feiner,[5] L Henke,[6] U Müller[1]

[1] Institut für Humangenetik, Justus-Liebig-Universität Giessen, Giessen, Germany; [2] Bioscientia, Zentrum für Humangenetik, Ingelheim, Germany; [3] Pädiatrie II, Universität Göttingen, Göttingen, Germany; [4] Neuropädiatrie, Universität Dresden, Dresden, Germany; [5] Neurologische Praxis, Tuttlingen, Germany; [6] Institut für Blutgruppenforschung LGC GmbH, Köln, Germany

Correspondence to:
Prof Dr Ulrich Müller, Institut für Humangenetik, University of Giessen, Schlangenzahl 14, D-35392 Giessen, Germany; Ulrich.Müller@humangenetik.med.uni-giessen.de

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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 equinovarus), 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 (BH_4), an essential cofactor of phenylalanine-, tyrosine- and tryptophan-hydroxylase. Therefore, depletion of BH_4 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 were 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 32 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 36 (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.[10] Of the *GCH1* deletion-positive patients, additional family members (particularly

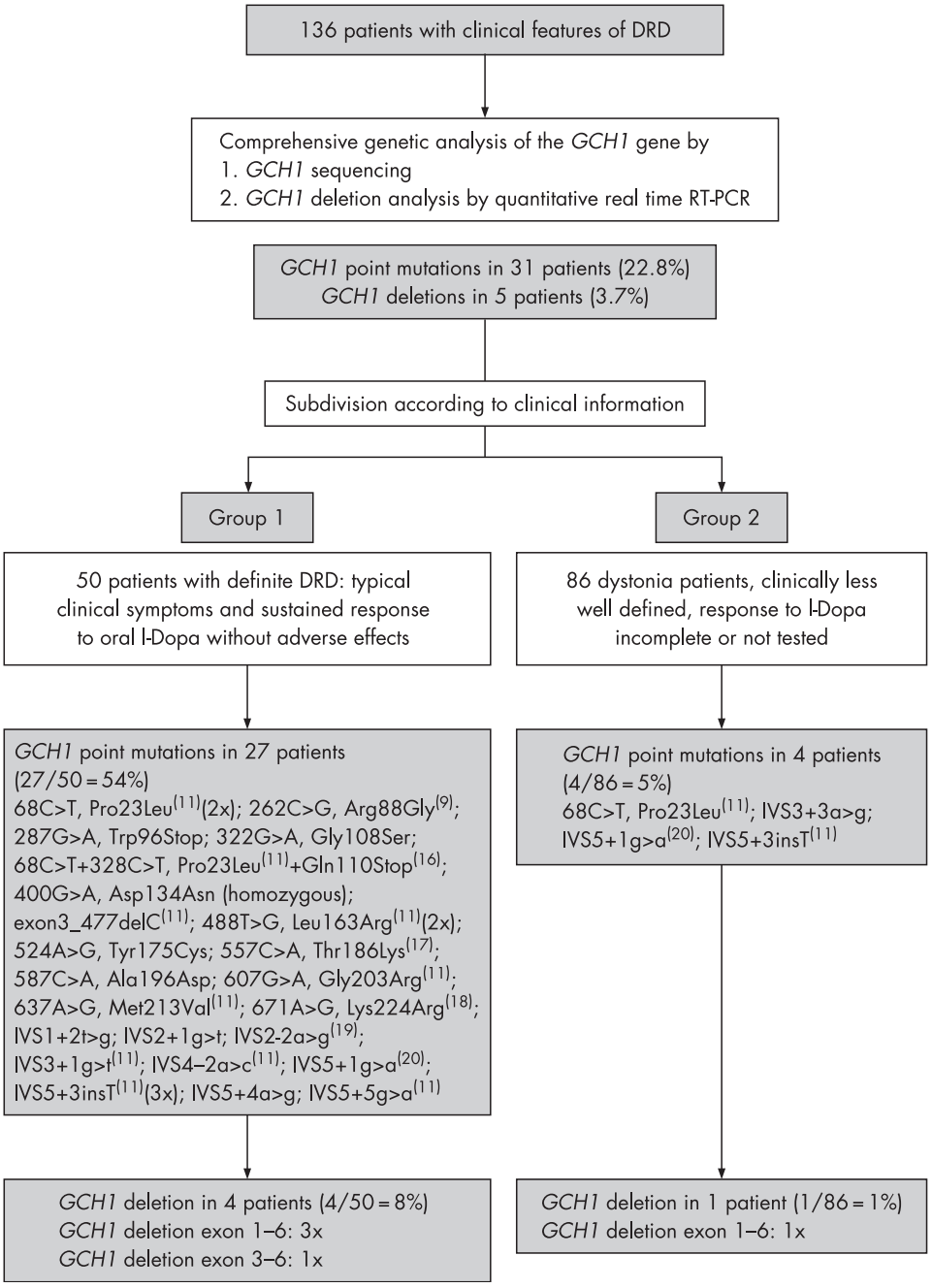


Figure 1 Flow-chart of *GCH1* sequence and deletion analysis in 136 patients with clinical features of Dopa-responsive dystonia (DRD). Numbers in brackets indicate references. For clinical features, see text ("Patients").

first-degree relatives) were also analysed for *GCH1* gene deletions.

Paternity testing by microsatellite analyses

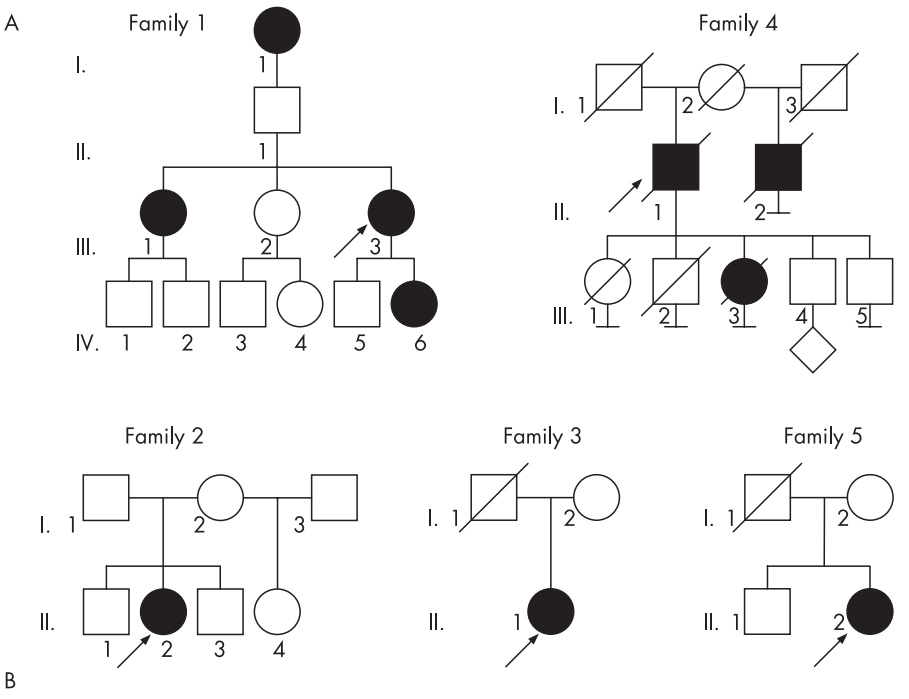
Paternity testing was performed in a family with an apparently *de novo* mutation of *GCH1*. A total of 28 autosomal and 5 X-chromosomal short tandem repeat loci were analysed.[12]

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



Family	Proband	*GCH1* ddCt values in real-time qPCR deletion analysis						*GCH1* deletion
		Exon 1	Exon 2	Exon 3	Exon 4	Exon 5	Exon 6	
1	II.1	1.02	1.08	0.66	0.79	1.29	1.15	Exons 1–6
	III.1	1.11	1.07	0.71	1.39	1.27	1.13	Exons 1–6
	IV.1	0.14	0.30	–0.23	0.18	0.11	0.00	No
	IV.5	–0.37	0.06	–0.22	–0.26	0.23	0.24	No
	IV.6	1.40	1.21	0.93	1.30	1.05	1.18	Exons 1–6
2	I.1	–0.26	–0.04	–0.05	0.15	–0.21	–0.21	No
	I.2	0.23	–0.09	–0.10	0.31	–0.36	–0.24	No
	II.2	0.96	0.83	1.01	1.29	0.73	1.18	Exons 1–6
3	II.1	1.38	0.91	–0.47	1.40	1.39	1.32	Exons 1–6
4	II.1	0.61	0.85	0.77	0.79	1.26	1.27	Exons 1–6
5	I.2	0.39	0.15	1.06	1.08	1.15	1.30	Exons 3–6
	II.1	–0.40	–0.27	–0.24	0.24	–0.20	–0.01	No
	II.2	–0.35	0.04	0.91	1.15	0.90	0.94	Exons 3–6

Figure 2 Pedigrees of the five families with deletions of *GCH1* (A) and summary of deletion analysis results (B). Index cases are indicated by arrows. (A) The four affected females of **family 1** had characteristic features of dopa-responsive dystonia (DRD). The first manifestation occurred in the legs during childhood (ages 5–10 years) and therapeutic response to L-Dopa was dramatic. The affected individuals tested (III.1 and IV.6) had a heterozygous *GCH1* deletion of exons 1 to 6. The same deletion was found in the asymptomatic male II.1 (obligate carrier). The *GCH1* gene was not mutated in asymptomatic individuals IV.1 and IV.5 (negative intra-familial controls). II.2, the only affected person of **family 2**, also had characteristic DRD with childhood onset of symptoms (gait disturbance and pes equinovarus) and subsequent generalisation, circadian fluctuation of symptoms and an excellent therapeutic response to L-Dopa. She had a *de novo* deletion of *GCH1* exons 1 to 6. The only affected person in **family 3** (II.1) also suffers from characteristic DRD. Onset of disease was in the legs during childhood; dystonia generalised in the course of several years. Therapeutic response to L-Dopa was satisfactory. She had a deletion of all six exons of *GCH1*. The proband II.1 of **family 4** developed gait disturbance and tremor of the hands during childhood. There was slow progression of neurological symptoms. He developed alcoholism and was treated for psychiatric disturbances. Treatment with L-Dopa was tried only briefly with 200 mg and was discontinued when no convincing improvement was achieved. He died at the age of 73 years in a nursing home and was never definitively diagnosed with DRD. The patient had a heterozygous *GCH1* deletion of exons 1 to 6. Additional family members were affected with similar symptoms but have not been available for genetic analysis. The only affected person of **family 5** had childhood-onset dystonia that started in the legs and subsequently generalised. DRD was diagnosed at the age of 26 years and she is now successfully being treated with L-Dopa. QPCR analysis revealed a partial *GCH1* deletion of exons 3 to 6 that the affected women had inherited from her asymptomatic mother. (B) Deleted *GCH1* exons with delta–delta cycle of threshold (ddCT) values between 0.6 and 1.4 are indicated by grey boxes in comparison with intra-familial controls without *GCH1* deletions (ddCT values between −0.4 and 0.4).

detected heterozygous deletions of *GCH1* in five patients. Four of these patients had been assigned to group 1 and one to group 2. The entire *GCH1* gene (exons 1 to 6) was deleted in four of the five patients. In one of these patients, *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 *GCH1* deletion was proven to be familial. One of these familial *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 *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 *GCH1* deletion (exons 1 to 6) was proven to have arisen *de novo* (patient II.2 of family 2; fig 2). Both parents had two copies of *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 *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 Gln110Stop, have not been previously reported. The relative frequency of 47% missense mutations is comparable to that described in the literature (55% missense mutations in the recent *GCH1* mutation review by Thöny and Blau[13]). In contrast, the frequency of 44% intronic splice-site mutations in our study is higher than previously described (13%[13]), whereas nonsense and frame-shift mutations occurred less frequently (6% and 3% versus 17%[13] and 15%,[13] respectively).

Deletion analysis was performed in all patients with no detectable point mutation. Of the five deletions found, four were detected in patients of group 1. One deletion had occurred *de novo* (family 2; fig 2) and is the first non-familial *GCH1* deletion reported in DRD to date.

The overall frequency of *GCH1* point mutations in clinically definite DRD patients documented in this study (54%) corresponds well with the 50–60% reported in the literature.[11 14 15] However, the frequency of deletions has not been studied systematically in large patient cohorts. There is only one report of the systematic screening for *GCH1* deletions in a small group of patients by Hagenah *et al*.[9] This study adopted rigorous clinical criteria and included 23 patients after positive L-Dopa testing. Two *GCH1* deletions were identified (2/23 = 8.7%). Here, we describe four *GCH1* deletions in 50 patients with clinically definite DRD. This corresponds to a deletion frequency of 8% in DRD patients and is consistent with the results obtained by Hagenah *et al*.[9]

The findings of most mutations in patients assigned to group 1 also corroborate that L-Dopa responsiveness in a dystonia patient is a strong clinical criterion for DRD. Comparatively low detection rates of *GCH1* point mutations and deletions were obtained in group 2, which was clinically less well defined and contained many dystonia patients in whom no systematic L-Dopa test was performed before molecular analysis. Therefore, group 2 may include patients with other forms of dystonia or early-onset parkinsonism.

The frequent occurrence of deletions of *GCH1* indicates that haploinsufficiency (reduced dosage) of GTPCH1 is the molecular cause of signs and symptoms in DRD. The lack of clear genotype–phenotype correlations in DRD for *GCH1* point mutations[11 14] further supports haploinsufficiency as the molecular basis of DRD. Genotype–phenotype correlations are also absent in patients with different deletions of *GCH1*. To date, seven complete *GCH1* deletions comprising all six *GCH1* exons (four in this publication; and three in previous publications[7 9 10]) and six partial *GCH1* deletions comprising different *GCH1* exons (*GCH1* exons 3–6 in this publication; exons 5 and 6, as well as exons 2–6[10]; exons 1–4[9]; and exon 1 and exons 1–3[8] 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.

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