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# Mutations of *GCH1* in Dopa-responsive dystonia

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**Summary.** Dopa responsive dystonia (DRD) is an autosomal dominant dystonia caused by mutations in the gene *GCH1* in about 50% of cases. *GCH1* codes for GTP cyclohydrolase I, a rate limiting enzyme in the synthesis of tetrahydrobiobterin ($BH_4$) from GTP. There is reduced penetrance and pronounced variation in expressivity of *GCH1* mutations in families with DRD. Correlations between given mutations in *GCH1* and phenotypes cannot be established. Mutations in *GCH1* appear to function as dominant-negatives but the exact mechanism remains unclear. Additional open questions in DRD include the molecular mechanisms resulting in highly variable expressivity of symptoms and the more likely occurrence of symptoms in a female than in a male carrier of a *GCH1* mutation.

**Keywords:** Dopa-responsive dystonia, DRD, Segawa syndrome, *GCH1*, pteridine.

## Introduction

Dystonias are movement disorders characterized by "involuntary, sustained muscle contractions affecting one or more sites of the body, frequently causing twisting and repetitive movements, or abnormal postures" (Ad hoc committee, 1987). Primary dystonias can be distinguished from secondary ones. While "secondary dystonia" refers to dystonia as one of several symptoms in the context of a neurological disorder, "primary dystonia" denotes a movement disorder with dystonia as the major or sole symptom (Fahn, 1984). Primary dystonias frequently occur in a familial pattern and are inherited as monogenic traits (Müller and Kupke, 1990). There are at least 9 autosomal dominant, one autosomal recessive and two X-linked recessive forms (Müller et al., 1998). Dystonias appear to be caused by abnormal function of the basal ganglia. Since there are no neuropathological anomalies in most forms, biochemical rather than morphological defects are considered the underlying

cause of the disorders. The elucidation of the genetic defect has confirmed this assumption in three forms of primary dystonia. In early-onset torsion dystonia (dystonia 1) mutations in a gene coding for an ATP-binding protein with homology to heat shock proteins (torsin A) cause disease (Ozelius et al., 1997) and mutations in the gene *GCH1* coding for GTP cyclohydrolase I (GTPCH I) cause autosomal dominant Dopa-responsive dystonia (DRD, dystonia 5; Ichinose et al., 1994). A second gene coding for an enzyme involved in the biosynthesis of dopamine, i.e. tyrosine hydroxylase, can be mutated in an autosomal recessive movement disorder that is primarily associated with infantile parkinsonism (van den Heuvel el al., 1998; Swaans et al., 2000). While the role of torsin A in normal and abnormal function of the basal ganglia is presently unclear, a wealth of investigations starting with the landmark findings of Arvid Carlsson (Carlsson, 1993), have clarified most aspects of normal biosynthesis and function of dopamine. This article reviews our current understanding of the role of *GCH1* mutations in insufficient biosynthesis of dopamine and the resulting development of autosomal dominant DRD.

## Clinical picture of DRD

Dopa-responsive dystonia (DRD), also referred to as Segawa syndrome or hereditary progressive dystonia with marked diurnal fluctuation (HPD), is an autosomal dominant primary dystonia (Segawa et al., 1971). Clinical manifestations of DRD vary widely and cover a broad spectrum of signs and symptoms ranging from generalized and focal dystonia via postural anomalies, parkinsonism, and subjective complaints to subtle signs only seen upon induction during clinical examination (Deonna et al., 1986; Nygaard et al., 1988; Nygaard, 1993; Furukawa et al., 1996; Bandmann et al., 1998; Steinberger et al., 1998, 1999). The generalized form is usually characterized by onset during childhood, frequently starting in the legs, and by diurnal worsening of symptoms in about 75% of cases. Although less commonly, adolescence- and even adulthood-onset can occur. In general, signs and symptoms are less severe in patients with a later age of onset. Frequently observed findings in generalized DRD include dystonia of the extremities, torticollis, blepharospasm, oromandibular dystonia, and postural anomalies such as scoliosis and hyperlordosis. Fully affected patients are wheelchair-bound within a few years of onset. Less severe cases present with focal dystonia such as abnormal positioning of one or of both feet sometimes resulting in fixed pes equinovarus. In other patients the main symptoms are rigidity of the legs and/or pretibial pain. In the least severely affected cases symptoms can only be induced by a simple writing test. When asked to write or draw with the left hand, discrete dystonic movements occur in the right hand and/or leg in a right-handed person. The reverse findings are observed in a left-handed person. During childhood DRD may present with a phenotype resembling atypical cerebral palsy (Nygaard et al., 1994, Bandmann et al., 1996). In adult-onset forms parkinsonism can be the main or only sign (Steinberger et al., 1998). Due to its highly variable expressivity, the diagnosis of DRD is difficult. There is, however, one hallmark of the disorder observed in most cases, i.e. a

dramatic therapeutic response of signs and symptoms to treatment with the dopamine precursor L-Dopa.

## Prevalence of DRD

DRD is a rare disorder and its prevalence is given as 0.5 per 1 million (Nygaard, 1993). This, however, is probably an underestimate for two reasons. First, the disorder has been more widely recognized only during the last 10 years or so and it is still not always correctly diagnosed. Secondly, many signs and symptoms are not severe enough for patients to seek clinical advice.

## Genetics of DRD

DRD is inherited as an autosomal dominant trait with reduced penetrance and highly variable expressivity of symptoms. Females are 2–4 times more frequently affected than males (Ichinose et al., 1994). Based on findings in one large family penetrance of fully expressed disease was given as approximately 30%. If minor symptoms were considered in this family, however, penetrance was estimated at 42% to 62% (Nygaard et al., 1990). Other estimates of penetrance are 15% in men and 45% in women (Nygaard et al., 1993a). Taking into account subtle signs and symptoms the penetrance may be close to 100% in females and approximately 60% in males. These numbers are based on clinical and molecular findings in 5 unrelated families with DRD (Steinberger et al., 1998).

Linkage analyses in 3 families have assigned the disease locus in DRD to the long arm of chromosome 14 (14q22.1–q22.2) (Nygaard et al., 1993b) and a candidate gene approach has identified *GCH1* as the disease gene in DRD (Ichinose et al., 1994).

## GCH1 and GTP cyclohydrolase I

*GCH1* is composed of 6 exons of 477 bp, 110 bp, 56 bp, 32 bp, 85 bp, 2,127 bp and spans approximately 30 kb of genomic DNA (GenBank accession numbers: Z30952, U19256, U19257, U19258, U19259, D38602). A full-length cDNA of 2,129 bp was cloned (Togari et al., 1992; Nomura et al., 1995). Its open reading frame is 750 bp and there appear to be several splice variants that differ at their 3′ ends. The full length transcript of *GCH1* (~4 kb on Northern blots) codes for the 250 amino acid subunit of GTP cyclohydrolase I, which appears to be a homodecamere in humans (Nar et al., 1995). GTP cyclohydrolase I is the rate limiting enzyme in the synthesis of tetrahydrobiopterin ($BH_4$) from GTP (Fig. 1) (Nichol et al., 1985). $BH_4$ is an essential cofactor for the three amino acid monooxygenases phenylalanine, tryptophan, and tyrosine hydroxylase which convert the three amino acids in tyrosine, serotonin, and L-Dopa (Fig. 1).

## GCH1 mutations

Homozygous mutations of *GCH1* have been recognized as a rare cause of hyperphenylalaninemia for some time (Niederwieser and Curtius, 1987;

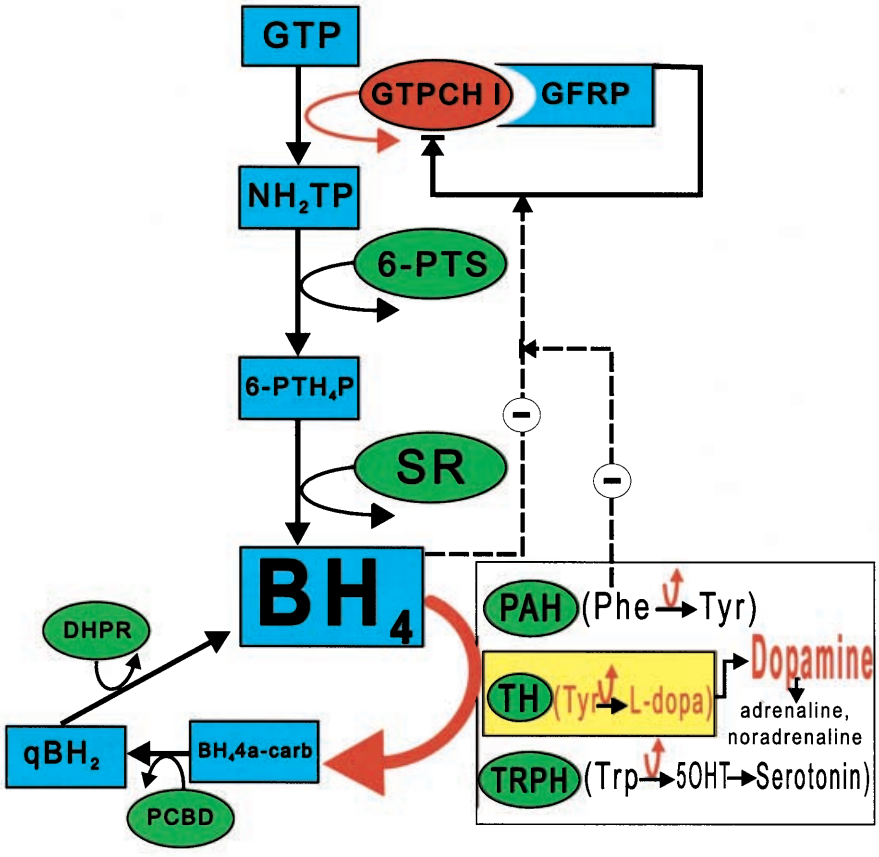


**Fig. 1.** Pteridine pathway. *GTP* Guanosine triphosphate, *GTPCHI* (GTP cyclohydrolase 1), *NH2TP* Dihydroneopterin triphosphate, *6-PTS* 6-Pyruvoyl tetrahydropterin synthase, *6-PTH4P* 6-Pyruvoyl tetrahydropterin, *SR* Sepiapterin reductase, *BH4* Tetrahydrobiopterin, *$BH_4$4a-carb* Tetrahydrobiopterin-4a-carbinolamine, *PCBD* Pterin-4a-carbinolamine dehydratase, *$qBH_2$* Quinoid dihydrobiopterin, *DHPR* Dihydropteridine reductase, *PAH* Phenylalanine hydroxylase, *GFRP* GTP cyclohydrolase I feedback regulatory protein, *TH* Tyrosinhydroxylase, *TRPH* Tryptophanhydroxylase, *Phe* Phenylalanine, *Tyr* Tyrosine, *Trp* Tryptophan, *5OHT* 5-OH-Tryptophan

Ichinose et al., 1995; Thöny et al., 2000). To date 16 cases have been described. Deficiency of $BH_4$ and the resulting decreased activities of phenylalanine, tryptophan, and tyrosine hydroxylase explain the clinical picture of this progressively deteriorating neurological disorder. Major symptoms include mental retardation, convulsions, disturbance of muscle tone with truncal hypotonia and hypertonia of the extremities, abnormal movements, difficulties in swallowing, hypersalivation, hyperthermia in the absence of infections and hyperphenylalaninemia refractory to phenylalanine restricted diet.

Heterozygous mutations of *GCH1* are the underlying cause of more than half of the cases of autosomal dominant DRD. Mutations have been detected in all six exons of the gene, and at intronic splice sites and have been summarized by Nishiyama et al. (2000). About 52% are missence, 17% are nonsense, 11% are splice site mutations, and 20% are deletions/insertions. Missense

mutations are thought to generate abnormal function of the enzyme by the exchange of an amino acid in the polypeptide and truncation of the protein is the result of nonsense, splice site, and frameshift mutations by deletion/insertion. The splice site mutations detected so far affect the consensus splice sites GT at the 5′ and AG at the 3′ ends of introns.

Mutations in *GCH1* result in reduced GTPCH I activity and thus in decreased synthesis of $BH_4$. This in turn compromises activity of tyrosine hydroxylase and dopamine synthesis. In contrast, the activity of the other two amino acid monooxygenases, i.e. phenylalanine and tryptophan hydroxylase is not sufficiently affected to cause symptoms. However, loading tests in carriers demonstrate reduced clearance of phenylalanine (Hyland et al., 1997). Apart from its role as a cofactor of amino acid monooxygenases, $BH_4$ also appears to increase the release of dopamine into neuronal synapses at high concentrations (Koshimura et al., 1994). Therefore, reduced levels of $BH_4$ might also affect the amount of dopamine in the basal ganglia directly. Circadian worsening of symptoms in DRD appears to be directly correlated with the amount of $BH_4$ available. While daytime activity requires dopamine and thus reduces $BH_4$ levels (half life 4.5 hours; Kapatos, 1990), even low amounts of GTPCH I activity are sufficient to at least partially restore the amount of $BH_4$ during nocturnal quiescence.

The activity of GTPCH I was shown to be reduced by more than 50% in PHA stimulated lymphocytes in several affected heterozygous mutation carriers (Ichinose et al., 1994) thus suggesting that mere haploinsufficiency is not the underlying cause of reduced synthesis of L-Dopa, dopamine, and the resulting DRD. It is likely that mutations have a dominant-negative effect. Thus the mutant polypeptide might form non- or dysfunctional heterodecameres with wild-type subunits of GTPCH I. Cotransfection experiments with wild-type and mutated *GCH1* cDNA support this notion (Hirano and Ueno, 1999). On the other hand, formation of heterodecamers of GTPCH1 could not be demonstrated for two mutations (R88W, R184H; Suzuki et al., 1999). In both cases, the aberrant subunits coded for by the mutated alleles appeared not be capable of interacting with the wild-type subunits. However, additional experiments are required to demonstrate directly the lack of formation of heterodecamers in these cases.

*GCH1* mutations might also exert a dominant-negative effect at the transcriptional level. For example, there is evidence that the relative levels of mutant *GCH1* mRNA contribute to enzymatic variation (Hirano et al., 1996). Furthermore, under physiological conditions normally occurring splice variants of *GCH1* might be involved in the regulation of gene expression. Of three species of human liver *GCH1* cDNAs one codes for the full length transcript and the other two code for inactive isoforms (Gütlich et al., 1994). It might be that the ratio of full-length to alternatively spliced transcripts determines the amount of functional GTPCH I subunits synthesized.

Reduced penetrance of DRD and less common occurrence of the disease in male than in female mutation carriers implies additional factors in the regulation of GTPCHI activity. Excess of DRD in females can be explained by physiologically lower levels of the enzyme in females as compared to

males. Findings by Shimoji et al. (1999) of lower levels of *GCH1* mRNA in brains from female than from male mice support this notion. Due to its lower base level, GTPCHI activity is more likely to drop below a critical level in female than in male mutation carriers. It will be interesting to find out whether different types of mutations show differences in the sex ratio of affecteds. One could speculate that mutations causing GTPCHI activity to decrease by only slightly more than 50% affect females only. Conversely, more severe mutations might affect both sexes equally. It is also not clear, yet, whether hormonal differences between the sexes account for lower GTPCHI activity in females than in males. If hormonal factors can influence expression of *GCH1* such differences might also account for both the variation in expressivity and the reduced penetrance observed in DRD. The study of additional genes ("modifier genes") in DRD will become possible once suitable mouse models are available. Introduction of the same mutation of *GCH1* in different isogenic strains will facilitate the identification of such genes and the investigation of their role in disease development.

## Conclusions

Mutations in *GCH1* are a common cause of autosomal dominant DRD. It is well established that these mutations cause disease by depletion of dopamine. However, the exact molecular mechanism resulting in decreased GTPCHI activity and reduced dopamine levels require further investigations. Furthermore, much remains to be done to understand the underlying molecular causes of reduced penetrance, highly variable expressivity and of female preponderance of DRD.

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