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Heterozygous mutation in 5'-untranslated region of sepiapterin reductase gene (*SPR*) in a patient with dopa-responsive dystonia

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Abstract The search for mutations in genes coding for components of the biopterin pathway other than *GTPCHI* revealed a mutation in the gene coding for sepiapterin reductase (*SPR*) in 1 of 95 patients with *GCHI*-negative dopa-responsive dystonia (DRD). The mutation detected in *SPR* is a G→A transition at position -13 of the untranslated region of the gene. This resulted in drastically reduced activity of sepiapterin reductase in the patient's fibroblasts. The findings indicate that haploinsufficiency of *SPR* can be a rare cause of DRD.

Keywords Dopa-responsive dystonia · Sepiapterin reductase · Biopterin metabolism · *GCHI*

Introduction

Primary dystonias are a genetically and clinically heterogeneous group of movement disorders [1]. Disease

genes have been identified in several primary dystonias, including childhood-onset dystonia (DYT-1), X-linked dystonia-parkinsonism (DYT-3) dopa-responsive dystonia (DYT-5), and myoclonic dystonia (DYT-11) [2, 3, 4, 5]. In many cases of dystonia, however, the underlying genetic defect remains unknown. The identification of additional disease genes in this group of disorders is—among other reasons—hampered by the lack of a sufficient number of large families available for study. Therefore, traditional positional cloning approaches, which are most commonly based on the chromosomal localization of the disease gene by linkage analyses in large families, need to be complemented by other methods such as the investigation of candidate genes. Candidate genes can be deduced from biochemical pathways implicated in some forms of primary dystonias. For example, the finding of mutations in *GCHI*, the gene coding for GTP cyclohydrolase I (GTPCH [E.C. 3.5.4.16]), the rate-limiting enzyme in biopterin synthesis, has allowed identification of additional genes that are potential candidates in some dystonias. These genes code for the various enzymes involved in the biosynthesis of biopterin (Fig. 1). One of these enzymes is sepiapterin reductase (SR [E.C.1.1.1.153]) that converts 6-pyruvoyl-tetrahydropterin (PTP) to tetrahydrobiopterin (BH₄) [6].

Homozygous mutations in the gene coding for SR (*SPR*) have been identified in two patients with various signs and symptoms including dystonia. In both cases the

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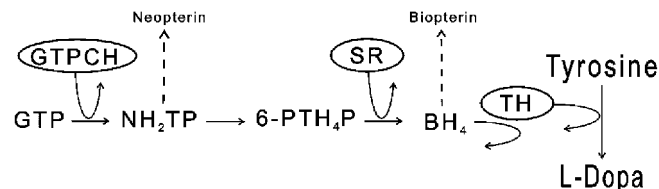


Fig. 1 Simplified pteridine pathway (GTP guanosine triphosphate, GTPCH GTP cyclohydrolase 1, NH₂TP dihydroneopterin triphosphate, 6-PTH₄P 6-pyruvoyl-tetrahydropterin, SR sepiapterin reductase, BH₄ tetrahydrobiopterin, TH tyrosine hydroxylase)

mutation rendered the enzyme inactive [7]. These findings prompted us to search for *SPR* mutations in patients with primary dystonias of unknown cause. We identified a unique heterozygous base change in the 5'-untranslated region of *SPR* in one patient that resulted in reduced activity of SR.

Case report

The patient is a 26-year-old woman. The history indicated that she walked on tiptoes during childhood, suggesting the development of fixed pes equinovarus. These symptoms disappeared before puberty. At the age of 15 years she noticed abnormal movements of the 4th and 5th digits of the left hand that were initially triggered by intended movements and later occurred spontaneously. At 19 years she developed gait anomalies with internal rotation, adduction, and extension of the left leg. The symptoms were aggravated by exertion. At 23 years dystonic movements occurred in the right leg and she noticed occasional tremor of the right arm. During pregnancy at 24 years of age symptoms improved and deteriorated drastically 2 months after delivery. Currently, the major findings are gait anomalies primarily caused by dystonia of the left leg, tremor of the left arm, dystonia of digits 4 and 5 of the left hand, and involvement of the vocal cords (intermittent voice tremor). There is a pronounced circadian fluctuation of symptoms. No family history could be obtained from the patient since she had been adopted and does not know her biological parents.

A positive response of signs and symptoms to L-dopa (up to 3×200 mg L-dopa in combination with 40 mg benserazide daily) was noticed in several trials of treatment. However, a continuous administration was not tolerated due to several adverse effects such as headache and nausea.

Materials and methods

DNA was extracted from peripheral blood of the patient according to standard procedures. A primary fibroblast culture was established from a skin biopsy of the patient.

Mutation analysis of *SPR*

In total 95 patients who presented with dystonia responsive to L-dopa and did not carry a mutation in *GCH1* were tested for mutations in *SPR*. All three exons of *SPR* including intron-exon boundaries were amplified by PCR. Primers and PCR conditions are given in Table 1. The amplification products were subjected to single-strand conformational polymorphism (SSCP) analysis [8]. Both strands of amplification products that resulted in a band-shift upon SSCP were sequenced according to standard procedures. In total 100 unaffected controls (200 chromosomes) were investigated for sequence changes found in given patients. In silico modeling of a putative wild-type and mutated transcript was performed as described previously [9].

Table 1 Primer sequences and PCR conditions for amplification of *SPR* exons analyzed

Exon	Designation primer	Primer sequence	Fragment length	Program			
1	FPE1-1	ACT GGG CTC CCG CCA CGG TTT GAC	233 bp	95° 10 min	36×		
	BPE1-1	CAG CCC GCC CTC CAT GCT CCT GTT		94° 70 s			
	FPE1-2	GCA CCG CCT CCT GCC TGG TCT CG		67° 30 s		35×	
	BPE1-2	CTC CAG CTG GCG CAG TGC CTC GTC		72° 30 s			
	FPE1-3	CCG CTC CTG GCC TCG CTG CTG TC		72° 7 min			38×
	BPE1-3	AGT GGG CGC TCA CAT GGG GAG TCC		94° 3 min			
	FPE 1-4	CAC CAG CAA CCA AGG GAA CCA GAT	94° 40 s	35×			
	BPE 1-4	TGC GGG CGC TAA GGA CAA G	70° 30 s				
			72° 20 s		34×		
			72° 7 min				
			94° 3 min			38×	
			94° 30 s				
		63° 30 s	38×				
		72° 20 s					
		72° 7 min		38×			
		94° 3 min					
		94° 30 s			38×		
		63° 30 s					
		72° 10 s	38×				
		72° 7 min					
		94° 3 min		38×			
		94° 40 s					
		56° 30 s			38×		
		72° 30 s					
		72° 7 min	38×				
		72° 7 min					

Biochemical studies

Primary skin fibroblasts were cultured in DMEM containing 10% fetal bovine serum plus penicillin and streptomycin. SR activity was tested in triplicate in unstimulated fibroblasts as described previously [10]. The enzyme activity was expressed as units per milligram of protein in cell lysate. Intracellular concentrations of neopterin and biopterin were measured after 24 h stimulation with cytokines [10]. Western blot analysis was performed using rabbit polyclonal anti-hSPR antibody (kindly provided by Dr. H. Ichinose, Japan) and quantified using the Appraise densitometry system (Beckman, USA).

Results

We tested 95 patients with dopa-responsive dystonia (DRD) and no mutation in *GCH1* for mutations in *SPR*. In one patient a G→A transition was detected at position -13 of the 5'-untranslated region of *SPR* (Fig. 2). This base change was neither detected in 100 unaffected controls (200 chromosomes) nor in the remaining 94 dystonia patients tested within this study. The findings indicate that the base change represents a potentially causative mutation rather than a rare neutral polymorphism.

Since a causative mutation in the 5'-untranslated region of *SPR* could affect the expression level of SR, we investigated SR activity in the patient's fibroblasts. SR activity was 62 $\mu\text{U}/\text{mg}$ and thus significantly reduced compared with controls (99–185 $\mu\text{U}/\text{mg}$). This was also demonstrated by western blot analysis using anti-SR antiserum. As shown in Fig. 3, the intensity of the SR band obtained with the patient's proteins was reduced to approximately 39% of the band produced by a comparable amount of protein from a control sample.

The concentration of biopterin was also reduced in the patient's fibroblasts compared with controls (82 pmol/mg vs. 154–303 pmol/mg). The neopterin concentration, however, was within the normal range (30 pmol in patient's fibroblasts compared with 18–98 pmol/mg in controls).

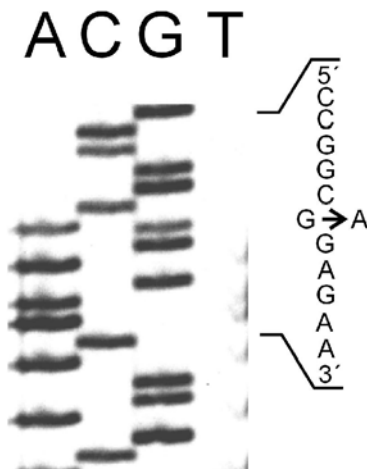


Fig. 2 Sequence analysis of the 5'-untranslated region of *SPR* of patient's DNA. The G→A transition is at position -13 of *SPR* (counting from ATG)

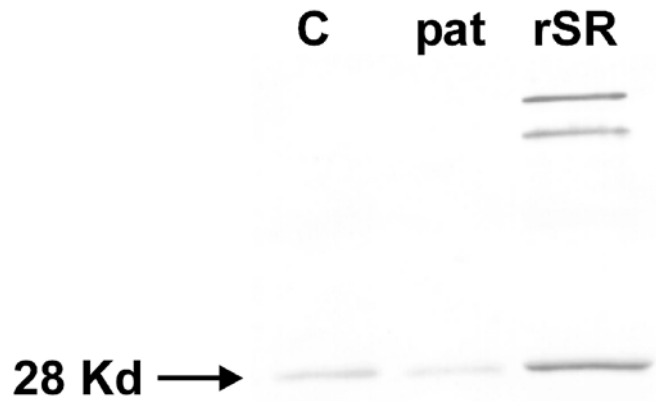


Fig. 3 Result of western blot analysis (C control, *pat* patient sample, *rSR* recombinant SR). Each sample contained 100 μg protein. For the band of the patient sample a signal reduced to approximately 39% compared with control was found. This indicates reduced expression of SR in the patient's fibroblasts

This indicates that the *GCH1* activity, alterations of which are the most-common cause of DRD, was normal.

The findings are consistent with the biological function of SR. The enzyme catalyzes conversion of PTP to BH_4 [6]. Reduction of its activity should also reduce the concentration of BH_4 , the production of which it catalyzes. This was observed in the patient. Conversely, the concentration of another intermediate of the biopterin pathway, neopterin, that is produced upstream of SR, remained unchanged in the patient.

Currently, it is not known how the mutation 13 bp upstream of the start codon of *SPR* affects expression of SR. The transcription start site of *SPR* appears to lie at position -81 [11]. Thus the mutation described here lies within a transcribed but untranslated region of the gene upstream from the start codon. It might be that the mutation interferes with formation of the proper secondary structure that is required for normal translation of this transcript. For example, in silico modeling suggests that an alternative folding of the transcript may occur in the presence of wild-type "G" at position -13 compared with an "A" as in the present patient (not shown).

The *SPR* mutation in the patient was present in the heterozygous state. This is consistent with autosomal dominant transmission of the trait. However, the patient's biological parents are not known and are thus not available for testing. Therefore, it remains unknown whether the mutation occurred de novo in the patient or whether it was transmitted through a parent. Furthermore, it requires the detection of *SPR* mutations in additional patients and families to determine whether penetrance is reduced as in individuals with *GCH1* mutations.

To our knowledge the present patient is the first who presented with a relatively mild form of DRD due to a heterozygous mutation in *SPR*. There are two additional published patients known with *SPR* mutations [7]. In these patients, however, the mutation was homozygous. Consistent with complete absence of SR activity in these patients, they were clinically much more severely affected

than the present patient. In addition to dystonia, the patients presented with microcephaly, growth and psychomotor retardation, spasticity, tremor, ataxia, oculogyric crises, marked diurnal fluctuations, and behavioral anomalies. To date five patients with homozygous SR deficiency have been tabulated in the BLODEF and BIOMDB databases (<http://www.bh4.org>), all with similar clinical signs. These patients carry mutations in exon 2 (Q119X, R150 fs, R150G, P163L) [12].

In conclusion, the investigation demonstrates that heterozygous mutations in genes coding for components of the biopterin pathway other than *GCHI* can cause DRD. In the present patient the mutation affected normal function of the gene encoding SR, which catalyzes the final step of BH₄ synthesis. The biochemical studies performed indicate that haploinsufficiency of *SPR* is the molecular pathological mechanism resulting in DRD in the patient.

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