c.207C>G mutation in sepiapterin reductase causes autosomal dominant dopa-responsive dystonia

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ABSTRACT

Objective: To elucidate the genetic cause of an Egyptian family with dopa-responsive dystonia (DRD), a childhood-onset dystonia, responding therapeutically to levodopa, which is caused by mutations in various genes.

Methods: Rare variants in all coding exons of *GCH1* were excluded by Sanger sequencing. Exome sequencing was applied for 1 unaffected and 2 affected family members. To investigate the functional consequences of detected genetic variants, urinary sepiapterin concentrations were determined by high-performance liquid chromatography.

Results: A heterozygous rare nonsynonymous variant in exon 1 of sepiapterin reductase (*SPR*, c.207C>G, p.Asp69Glu) was found in all affected family members. Urinary concentrations of sepiapterin were above the standard of normal controls in most *SPR* mutation carriers, suggesting functional biochemical consequences of the mutation. Variant filtering of all genes involved in the tetrahydrobiopterin pathway, required for levodopa synthesis, revealed an additional common variant in dihydrofolate reductase (*DHFR*, rs70991108). The presence of both variants was significantly stronger associated with the biochemical abnormality and the clinical disease state as opposed to 1 variant only.

Conclusions: The rare *SPR* mutation can cause autosomal dominant DRD with incomplete penetrance. The common *DHFR* variant might have synergistic effects on production of tetrahydrobiopterin and levodopa, thereby increasing penetrance. *Neurol Genet* 2017;3:e197; doi: 10.1212/NXG.0000000000000197

GLOSSARY

DHFR = dihydrofolate reductase; **DRD** = dopa-responsive dystonia; **MAF** = minor allele frequency; **NADPH** = nicotinamide adenine dinucleotide phosphate; **SPR** = sepiapterin reductase.

Dopa-responsive dystonia (DRD) encompasses a group of movement disorders with heterogeneous clinical and genetic manifestations, presenting with childhood-onset limb dystonia and response to levodopa therapy.[1] Additional neurologic presentations may include parkinsonian and pyramidal features.[2,3] Variants in the gene of GTP cyclohydrolase I (*GCH1*), a key protein of the tetrahydrobiopterin (BH$_4$) pathway, required for the biosynthesis of levodopa (figure, A), are the most frequent cause of autosomal dominant DRD, also known as DYT5a.[1,4] Variants of sepiapterin reductase (*SPR*), encoding another key protein of the BH$_4$ pathway (figure, A), have been identified in autosomal recessive DRD.[5] At present, only 1 patient with DRD without family history has been identified with a heterozygous *SPR* variant.[6] Here, we describe the first DRD family carrying a rare *SPR* variant (c.207C>G, p.Asp69Glu, chr2:73114768_C>G,
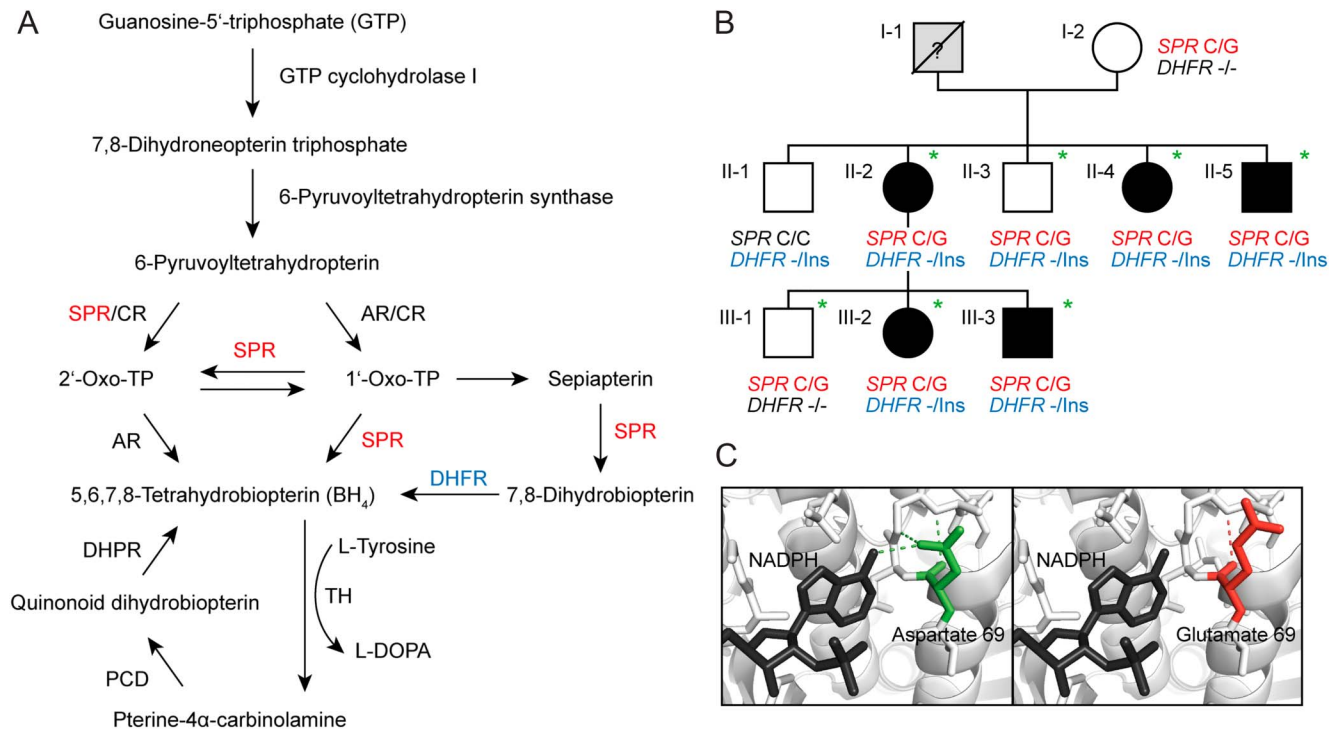

Figure Sepiapterin reductase and dihydrofolate reductase in the context of dopa-responsive dystonia

(A) Synthesis and regeneration of 5,6,7,8-tetrahydrobiopterin (BH$_4$), an essential cofactor of L-DOPA synthesis (scheme adapted from references 3, 11). Proteins of relevance are highlighted in red and blue. AR = aldose reductase; CR = carbonyl reductase; DHPR = dihydropteridine reductase; PCD = pterin-4α-carbinolamine reductase; TH = tyrosine hydroxylase; 2'-Oxo-TP = 1'-hydroxy-2'-oxopropyltetrahydrobiopterin; 1'Oxo-TP = 2'-hydroxy-1'-oxopropyl-tetrahydrobiopterin. (B) Pedigree of the investigated dopa-responsive dystonia family. Sepiapterin reductase (SPR) C/G = chr2:73,114,768 C/G in exon 1 of SPR; dihydrofolate reductase (DHFR)-/Ins = chr5:79,950,163-/TGGCGCGTCCCGCCCAGGT (19 base-pair insert) intronic, located upstream of exon 5 of DHFR. Green asterisks indicate family members with urine sepiapterin values slightly above the concentration range measured in normal controls. (C) Structure of the NADP-binding site in chain A of SPR obtained from the Research Collaboratory for Structural Bioinformatics protein data bank (identifier = 4XWY) in wild-type (left) and mutant states (right). Aspartate on position 69 forms hydrogen bonds (green dashed lines) with cofactor nicotinamide adenine dinucleotide phosphate (NADPH) (colored in black). In the case of a substitution of aspartate with glutamate, a loss of hydrogen bonds is predicted (dashed red line).

undetected in 8,599 alleles annotated in the Exome Variant Server) with autosomal dominant inheritance and incomplete penetrance.

METHODS Standard protocol approvals, registrations, and patient consents. Affected and nonaffected individuals of a family of Egyptian origin were examined by neurologists specialized in movement disorders (A.S.S. and G.U.H.) at the Department of Neurology, Ain Shams University, Cairo, Egypt. Ethical approval was obtained at Ain Shams University (RP/42) and Technical University of Munich (203/15s). All participants provided written informed consent. Consanguinity was excluded by interview and genetic analyses.

Sanger and next-generation sequencing. After excluding known rare variants in all 6 coding exons of *GCH1* by Sanger sequencing, exome sequencing was applied to 1 unaffected and 2 affected family members. Genomic DNA libraries were captured using the Nextera Rapid Capture Expanded Exome Kit (Illumina, San Diego, CA), and DNA fragments were sequenced on an Illumina HiSeq2000 system. Variants were identified by a standard analysis pipeline.

Variant filtering and validation. Variants with a minor allele frequency (MAF) >0.1%, based on >6,500 (The National

Heart, Lung, and Blood Institute Exome Sequencing Project, version ESP6500siv2), were discarded. Assuming a dominant disorder, only nonsynonymous variants were retained as candidate alleles, including missense, nonsense, stop-loss, and splice-site mutations, as well as small insertions and deletions present in the heterozygous state and present in both affected sisters (figure B, II.2 and II.4). Hypothesis-based variant filtering was applied to all genes in the SPR pathway, also considering variants with an MAF >1.0%. Candidate variants were validated by Sanger sequencing and cosegregation with disease in the family (table 1).

In silico analyses. The effects of amino acid substitutions on protein function were predicted using MutationTaster (mutationtaster.org), PolyPhen-2 (genetics.bwh.harvard.edu/pph2), and CADD (cadd.gs.washington.edu). Mutagenesis analysis was performed with PyMOL (pymol.org) based on the SPR protein structure 4XWY (PDB file). Functional protein domains were analyzed using HaploReg v4.1 (broadinstitute.org/mammals/haploreg/haploreg.php).

Urinary sepiapterin. First-void urine was collected, protected from light, immediately placed on dry ice, and stored at −80°C. For sepiapterin quantification, samples were prepared as described[7] and subjected to high-performance liquid chromatography analysis on a C18 reversed-phase column (Spherisorb ODS1, 5 μm, ID 4.6 mm, L250 mm; Waters, Milford, MA) and isocratic elution with 24 mM KH$_2$PO$_4$ (pH 5.0)/CH$_3$OH

Table 1 Primer sequences for the *SPR* gene encoding sepiapterin reductase (SPR) and for rs70991108 located in the *DHFR* gene encoding dihydrofolate reductase (DHFR)

Oligo name	Sequence	Orientation	Primer length	Tm	GC content, %	Length product	Gene	Annealing temperature, °C
SPR_Ex1_F	CCCGCCGACACCCGTA	Forward	16	61.19	75	466	SPR	59.9
SPR_Ex1_R	CCCTTAAATTCCCCAGCGGT	Reverse	20	60.03	55			
SPR_Ex2_F	GCATGGGAAGAGGATATCTGG	Forward	21	57.65	52.38	462	SPR	59.9
SPR_Ex2_R	GGACTAGGCCGCTTGC	Reverse	16	57.21	68.75			
SPR_Ex3_F	ACCCCCGACATAAAACAGGG	Forward	20	59.67	55	473	SPR	55.3
SPR_Ex3_R	CACAGCACAGACTCCTGACA	Reverse	20	59.61	55			
DHFR_rs70991108_F	AGAAAAGGGGAATCCAGTCG	Forward	20	57.21	50.00	473	DHFR	60.4
DHFR_rs70991108_R	GAGACATGGCAGGGCAAG	Reverse	18	58.08	61.11			

(85:15, vol/vol) (flow rate 1.1 mL/min over 15 minutes). A fluorescence detector (RF 20A XS; Shimadzu, Kyoto, Japan; excitation λ = 425 nm; emission λ = 530 nm) was used. Sepiapterin eluted with a retention time of 9.9 minutes. Urine samples were masked before analysis. Mean values of 4 technical measurements are presented.

Statistical analyses. Descriptive analyses and χ^2 statistics were calculated using Statistica 8.0 (StatSoft Inc., Tulsa, OK). Data are presented as mean ± SEM. $p < 0.05$ was considered statistically significant.

RESULTS Clinical description. We examined 5 affected and 4 nonaffected family members (figure, B). Symptom onset was between childhood and puberty (table 2). Patient II.4 presented with lower limb dystonia (tiptoe walking) and rigidity since childhood, with progressive course and diurnal variation. He improved markedly with L-DOPA, however with low-threshold drug-induced dyskinesia. His current medication comprises fractionated L-DOPA doses (750 mg/d), anticholinergics, and amantadine (400 mg/d). The other affected individuals showed diurnal lower limb dystonia, rigidity, and mild pyramidal signs. Patients (II.2 and II.5) experienced symptomatic benefit with L-DOPA and continued on pramipexole. Patients III.2 and III.3 were not treated with L-DOPA because of their young age, mild symptoms, and fear of their parents of L-DOPA–induced dyskinesia.

Genetic analysis. We found a nonsynonymous heterozygous variant in exon 1 of *SPR* (NM_003124.4 c.207C>G, p.Asp69Glu, genomic [GRCh37]: chr2:73114768_C>G) in all affected family members. In SPR, aspartate 69 is involved in binding the cofactor nicotinamide adenine dinucleotide phosphate (NADPH). In silico mutagenesis analysis predicted that the longer and more flexible side chain of glutamate 69 in mutation carriers leads to an increased distance and loss of hydrogen bonds to NADPH (figure, C).

Structural gene/exon variations of *SPR* were checked in the 3 samples in which whole-exome sequencing was performed using the BreakDancer tool (github.com/genome/breakdancer).[8] We did not detect such an event near *SPR* or elsewhere on chromosome 2.

In addition, we found an intronic 19 base-pair insertion (rs70991108) in an active transcription start site of the dihydrofolate reductase (*DHFR*) gene in all affected family members (table 2). This variant alters 8 regulatory motifs (HaploReg).

Urinary sepiapterin. SPR deficiency increases urinary sepiapterin levels.[7] To investigate the consequences of the variants observed in this study, we measured urinary sepiapterin concentrations in all members (table 2). All clinically affected family members had sepiapterin concentrations slightly above the range measured in normal controls (mean ± SD: 4.2 ± 3.12 μmol/mol creatinine), but below the values in proven SPR-deficiency patients (727 ± 230 μmol/mol creatinine).[7]

Genotype-phenotype relationship. The family member without the *SPR* variant (II.1) had normal sepiapterin concentrations and was healthy. From the 8 *SPR* variant carriers (table 2), 7 had elevated sepiapterin concentrations; 5 of those were clinically affected.

From 2 members without the *DHFR* variant (I.2 and III.1), 1 had elevated sepiapterin values, but both were healthy. From 7 *DHFR* variant carriers (table 2), 6 had elevated sepiapterin concentrations; 5 of those were clinically affected.

Six family members carried both *SPR* and *DHFR* variants (table 2); all of them had elevated sepiapterin concentrations; 5 of them were clinically affected.

There was a significantly stronger association of both variants simultaneously with the biochemical abnormality (χ^2 5.1429, $p < 0.05$) and the clinical disease state (χ^2 5.625, $p < 0.05$) as opposed to only 1 variant.

Table 2 Clinical, genetic, and biochemical data of the investigated DRD family

Family member	Sex	Age at examination, y	Dystonia	Disease onset	Phenomenology (in chronological order)	Response to L-DOPA	Variant SPR	Variant DHFR	Sepiapterin (µmol/mol Cr) ± SEM
I.1	M	ND	NA	NA	NA	NA	NA	NA	NA
I.2	F	56	−	−	−	ND	+	−	3.75 ± 0.63
II.1	M	35	−	−	−	ND	−	+	2.99 ± 0.53
II.2	F	32	+	Childhood	Dystonic posturing of both feet; with diurnal fluctuation, mild lower limb bradykinesia and rigidity, and extensor planter responses	+	+	+	15.44 ± 2.07[a]
II.3	M	29	−	−	−	ND	+	+	8.47 ± 0.72[a]
II.4	F	27	+	Childhood	Initially; lower limb dystonia, bradykinesia, and rigidity with diurnal variation progressed to generalized parkinsonism; L-DOPA–induced dyskinesia and pyramidal signs	+	+	+	28.43 ± 4.02[a]
II.5	M	21	+	Puberty	Lower limb dystonia (tiptoe walking) after prolonged walking; bilateral lower limb rigidity, postural tremor, and equivocal Babinski sign	+	+	+	7.39 ± 0.88[a]
III.1	M	14	−	−	−	ND	+	−	17.74 ± 3.92[a]
III.2	F	10	+	Childhood	Tiptoe walking with activity; fast fatigability with dystonic posturing on the left foot	ND	+	+	22.67 ± 4.75[a]
III.3	M	5	+	Infancy	Tiptoe walking; equinovarus deformity	ND	+	+	19.95 ± 2.40[a]

Abbreviations: Cr = creatinine; DHFR = dihydrofolate reductase; DRD = dopa-responsive dystonia; NA = not available; ND = not determined; SPR = sepiapterin reductase; − = not present; + = present; variant DHFR = chr5:79,950,163-/TGGCGCGTCCCGCCCAGGT (19 base-pair insert); variant SPR = chr2:73,114,768 C/G in exon 1.
Normal range of urine sepiapterin: 4.2 ± 3.12 µmol/mol Cr (mean of 10 controls without mutations in genes involved in the synthesis of L-DOPA).
[a] Elevated values.

DISCUSSION We identified a rare heterozygous variant of *SPR* in a family with DRD segregating as autosomal dominant trait (figure, B and table 2). *SPR* is essential for levodopa synthesis. In silico analysis suggested pathogenicity of this variant. Consistently, we found slightly elevated urine sepiapterin concentrations in 7 variant carriers, 5 of these presenting clinical symptoms. Since also 3 nonaffected siblings carried this variant, incomplete penetrance was assumed. Other *SPR* variants are known to cause autosomal recessive DRD. A heterozygous *SPR* variant in a single patient with DRD without family history had already indicated the possibility of autosomal dominant causality of *SPR* variants.[6] These observations suggest that this *SPR* variant has autosomal dominant pathogenicity with incomplete penetrance.

In the same family, we also identified a *DHFR* variant (rs70991108). In silico analysis suggested a potential effect of this variant on protein expression. Consistently, we found elevated urine sepiapterin concentrations in 6 and clinical symptoms in 5 of the 7 variant carriers. Two nonaffected siblings carried the variant as well. Because of its relatively high

allele frequency in the general population,[9] we do not assume this variant to be pathogenic per se.

Of interest, the presence of the common *DHFR* variant in addition to the *SPR* variant significantly increased the risk of the presence of the biochemical abnormality (elevated sepiapterin urine concentrations) and of clinical symptoms. The 1 sibling carrying both variants (*SPR* and *DHFR*) without clinical DRD symptoms might be either in a preclinical disease stage or might be protected by unknown resilience factors.

While high DHFR expression in the liver may account for a modest biochemical phenotype in the periphery, the very low cerebral DHFR expression might be further reduced by the variant, leading to a clinically significant disturbance of L-DOPA synthesis.[10] This suggests that the presence or absence of the common *DHFR* variant affects the penetrance of the autosomal dominant *SPR* variant. With regard to the sequential metabolic function of the *SPR* and *DHFR* gene products in the L-DOPA synthesis pathway (figure, A), this interpretation appears plausible. The *SPR-DHFR* pathway metabolizes sepiapterin to generate tetrahydrobiopterin, essential for the

formation of L-DOPA. The enzymes SPR and DHFR are serially catalyzing this reaction. Homozygous mutations affecting *SPR* on both homologous chromosomes impose a massive bottleneck within this pathway and are known to cause DRD. A heterozygous mutation affecting *SPR* on only 1 chromosome imposes a minor bottleneck for this pathway. The clinical penetrance of this condition may thus depend on additional cofactors affecting this pathway, such as epigenetic silencing of the healthy *SPR* allele. In our family, this cofactor seems to be the co-occurrence of an additional variant in the downstream enzyme *DHFR*, causing a "tandem stenosis" within this pathway.

Taken together, the present study proposes the rare *SPR* c.207C>G variant as a cause of autosomal dominant DRD with incomplete penetrance and the common *DHFR* rs70991108 variant as a potential modifier, significantly affecting the penetrance of the *SPR* variant.

AUTHOR CONTRIBUTIONS

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DISCLOSURE

REFERENCES

1. Klein C. Genetics in dystonia. Parkinsonism Relat Disord 2014;20(suppl 1):S137–S142.
2. Wijemanne S, Jankovic J. Dopa-responsive dystonia—clinical and genetic heterogeneity. Nat Rev Neurol 2015; 11:414–424.
3. Friedman J, Roze E, Abdenur JE, et al. Sepiapterin reductase deficiency: a treatable mimic of cerebral palsy. Ann Neurol 2012;71:520–530.
4. Müller U. The monogenic primary dystonias. Brain 2009; 132:2005–2025.
5. Bonafe L, Thony B, Leimbacher W, Kierat L, Blau N. Diagnosis of dopa-responsive dystonia and other tetrahydrobiopterin disorders by the study of biopterin metabolism in fibroblasts. Clin Chem 2001;47:477–485.
6. Steinberger D, Blau N, Goriuonov D, et al. Heterozygous mutation in 5'-untranslated region of sepiapterin reductase gene (SPR) in a patient with dopa-responsive dystonia. Neurogenetics 2004;5:187–190.
7. Carducci C, Santagata S, Friedman J, et al. Urine sepiapterin excretion as a new diagnostic marker for sepiapterin reductase deficiency. Mol Genet Metab 2015;115: 157–160.
8. Fan X, Abbott TE, Larson D, Chen K. BreakDancer: identification of genomic structural variation from paired-end read mapping. Curr Protoc Bioinformatics 2014;45:15.6.1–15.6.11.
9. Dutta S, Chattopadhayay S, Dasgupta C, Sarkar S. Low molecular weight heparin: a practical approach in deep venous thrombosis in palliative care. Indian J Palliat Care 2011;17:143–145.
10. Blau N, Bonafe L, Thony B. Tetrahydrobiopterin deficiencies without hyperphenylalaninemia: diagnosis and genetics of dopa-responsive dystonia and sepiapterin reductase deficiency. Mol Genet Metab 2001;74: 172–185.
11. Neville BG, Parascandalo R, Farrugia R, Felice A. Sepiapterin reductase deficiency: a congenital dopa-responsive motor and cognitive disorder. Brain 2005;128: 2291–2296.