


CASE REPORT

Detection of a new deleterious *SGCE* gene variant in Moroccan family with inherited myoclonus–dystonia

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Abstract

Myoclonus–dystonia (M-D) is a pleiotropic neuropsychiatric disorder with autosomal dominant mode of inheritance with variable severity and incomplete penetrance. Pathogenic variants in ξ -sarcoglycan gene *SGCE* are the most frequently known genetic cause of M-D with maternal imprinting, and in most cases, a symptomatic individual inherits the pathogenic variant from his or her father. This work reported a missense mutation c.662G>T inherited in the M-D Moroccan family described for the first time, which is deleterious based on protein modeling analysis.

KEYWORDS

c.662G>T mutation, familial myoclonus–dystonia, modeling, Morocco, *SGCE* gene

1 | INTRODUCTION

Myoclonus–dystonia (M-D) is a hyperkinetic movement disorder defined as a syndrome of sustained involuntary muscle contractions (myoclonus) frequently causing repetitive twisting movements or abnormal postures

(dystonia).¹ The myoclonic jerks typical of M-D most often affect the neck, trunk, and upper limbs with less common involvement of the legs. Approximately 50% of affected individuals have additional focal or segmental dystonia, presenting as cervical dystonia and/or writer's cramp.² M-D is compatible with an active life of normal span. Psychiatric

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disorders have been reported to be associated with M-D, obsessive-compulsive disorder (OCD), anxiety-related disorder, depression, and alcohol dependence. Most affected adults report a dramatic reduction in myoclonus in response to alcohol ingestion. Symptom onset is usually during the two first decades: earlier (<10 years) or later (up to 40 years).²

Myoclonus-dystonia is transmitted in an autosomal dominant manner and may occur sporadically. A proband with M-D may have inherited the disorder from a parent (50% chance of inheriting the pathogenic variant) or have the disorder as a result of a de novo pathogenic variant. The *SGCE* gene is imprinted, with incomplete penetrance, which is dependent on the parental origin, and occurs only when mutations affect the paternal copy of this gene. Thus, almost all children who inherit an *SGCE* pathogenic variant from their father develop symptoms; however, nearly 95% of children who inherit an *SGCE* pathogenic variant from their mother do not.²⁻⁴ There are several other human genes that are imprinted, including genes located on chromosome 7, and many of them are involved in human diseases. Usually, the mechanism of imprinting is a methylation of cytosine residues at the promoter region that inactivates the gene. This mechanism has been confirmed for the *SGCE* gene by showing a differential pattern of methylation of the parental allele in patients with M-D.³ In some of these patients, a loss of imprinting with subsequent biallelic expression of the *SGCE* gene has been demonstrated.⁴

The primary M-D locus identification has been carried out in a large North American family in 1999, and the locus was mapped on chromosome 7q21.3^{5,6} and confirmed in other families.^{7,8} The *SGCE* gene consists of 13 exons (exons 1–11, 11b, and 12) and encodes for a 438-amino acid protein with a single transmembrane domain. The *SGCE* is a member of a gene family that also includes α , β , δ , ϵ , and ξ sarcoglycans that constitute an essential structure of dystrophin-associated glycoprotein complex in striated muscle.

Mutations in the ξ -sarcoglycan gene (*SGCE*, DYT11 locus, and MIM 604149) represent the major genetic cause, but not the only one; other loci such as DYT15 on chromosome 18p11 are associated with the disease, and in a proportion of patients, no genetic alteration is found.⁹ Various mutations (>100) in the *SGCE* gene have been found to cause M-D, including nonsense, missense, deletions,¹⁰ and insertions.⁷ Most of these mutations lead to an abnormally short, nonfunctional ξ -sarcoglycan protein that is quickly broken down.^{11,12} Other mutations prevent the protein from reaching the cell membrane where it is needed. This lack of functional protein seems to affect the regions of the brain involved in coordinating and controlling movements and leads to the involuntary

movement's characteristic of myoclonus-dystonia. It is unclear why *SGCE* gene mutations seem to affect only these areas of the brain.¹³

The epidemiology of M-D is not well established. However, it is known that M-D affects most, if not all, racial groups including Africans, Europeans, Chinese, Indians, and Brazilians^{14,15} and it is clinically consistent across ethnicities. In Morocco, few studies have been conducted to explore *SGCE* gene mutations causing myoclonus-dystonia (M-D) and only two different heterozygous *SGCE* mutations (c.769A>C and c.391–3 T> C) have been reported in a study including 12 patients with a sporadic M-D.¹⁶ Thus, we include in this work the first observation of a familial form of myoclonus-dystonia, the case of a 17-year-old Moroccan girl suffering from myoclonus-dystonia, using a new approach for medical genetic counseling in the National Reference Laboratory (LNR) based on whole-exome sequencing (CentoXome Gold[®]) diagnostic strategy subcontracted by Centogene Company. Molecular explorations in neurogenetics using WES have a positive impact on the patient, and his family's medical care management and genetic counseling would help to the better comprehension of the pathogenicity of mutations.

2 | PATIENTS AND METHODS

2.1 | Patients and clinical evaluation

We report the case of a 17-year-old Moroccan girl suffering from myoclonus-dystonia, whose first symptoms began since her childhood: essential resting myoclonuses and actions accentuated by stress and emotion with a slight psychomotor retardation. The patient was operated at the age of two years for ventricular communication with good progress. A video EEG has been conducted at the age of 13 showing the absence of seizure activity related to epilepsy.

The disease is segregated in this Moroccan family in autosomal dominant form on the paternal side (6 cases) and over three generations (Figure 1), with incomplete penetrance. The parents are phenotypically normal. Cytogenetic examination showed a normal karyotype. The genetic study was approved by the ethics committee of Cheikh Khalifa Hospital according to the Declaration of Helsinki protocol, and all subjects gave written informed consent before testing.

2.2 | Methods

Blood samples were taken from the daughter, father, and mother. Blood samples were either deposited on a

paper for exome analysis subcontracted by *Centogene* Company or extracted using a Maxi-QIAamp DNA Extraction Kit (Qiagen, Hidden, Germany) according to the manufacturer's instructions. DNA was quantified using Qubit (Thermo Fisher Scientific). *SGCE* primers for exon 5 were designed using the Primer 3 software, ver 4.1.0 (F: 5'-CCTCTGATGAGCCTTGGATT-3' and R: 5'-TTCACAGACCAGGAAGCTTGAGA-3'). PCRs were conducted using 100 ng DNA using Platinum Hot Start PCR Master Mix (2X) on a Veriti thermal cycler (Thermo Fisher Scientific) using cycling conditions (95°C for 7 min; 35 cycles of 95°C for 30 s, 58°C for 40 s, and 72°C for 1 min; and 72°C for 15 min). PCR amplification product was controlled on 2% agarose gel.

For Sanger sequencing, the PCR product was purified using the ExoSAP method and sequenced using forward and reverse primers using a BigDye Terminator v3.1 Sequencing Kit (Applied Biosystems), and was run on a 3500 ABI sequencer (Applied Biosystems, Foster City).

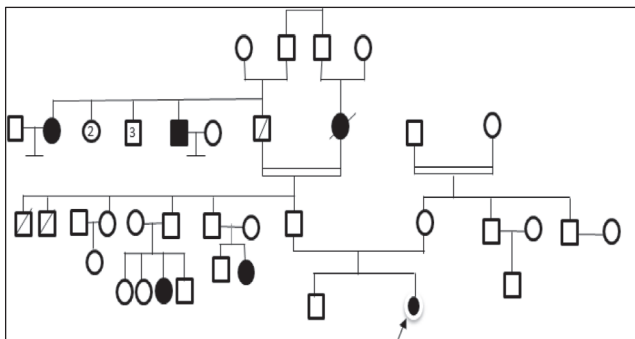
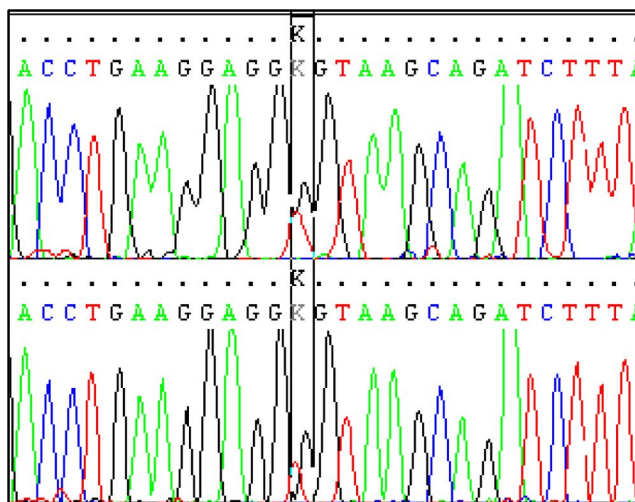
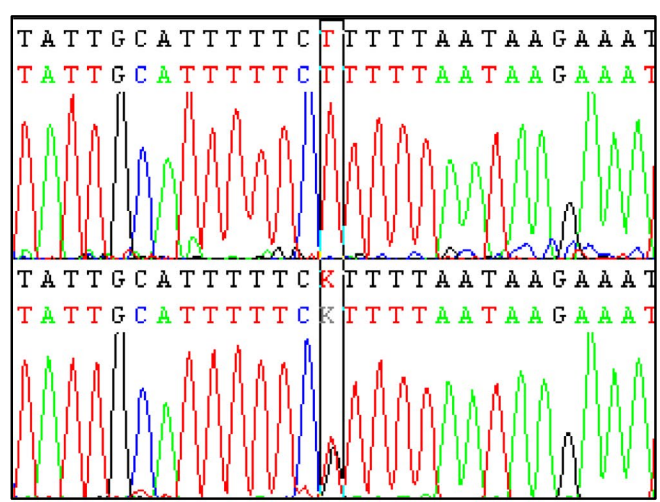


FIGURE 1 Pedigree of family with familial myoclonus-dystonia



(A) c.662G>T



(B) c.662G+99>T

FIGURE 2 Electropherogram showing the *SGCE* c.662G>T mutation (A), in father and daughter, and c.662G+99>T

Sequence analysis was performed using the SeqScape software v2.5 (RefSeq consensus sequence: NM_003919).

2.3 | Molecular modeling analysis

Molecular modeling analysis was performed to predict the effect of the c.662G>T = p. Gly221Val mutation found on the *SGCE* protein structure. The I-TASSER server¹⁷ was used to predict the 3D structure of the *SGCE* protein. The PyMOL v1.7.4 software was used to mutate the 221 amino acid. Then, both proteins (native and mutated) were minimized using the YASARA Energy Minimization Server.¹⁸ The change in protein stability upon p. Gly221Val mutation was estimated using CUPSAT,¹⁹ mCSM,²⁰ SDM,²¹ DUET,²² and DeepDDG programs.²³ The YASARA v20.8.23 software²⁴ was used for protein structure visualization.

3 | RESULTS AND DISCUSSION

3.1 | Whole-exome sequencing and Sanger sequencing

Through the use of a new whole-exome sequencing (CentoXome Gold[®]) diagnostic strategy, results show in exon 5 a new heterozygous mutation c.662G>T in the *SGCE* gene in the index case. The father also wears the same variant, so the transmission is subject to parental imprinting. The mother does not carry the variant. The *SGCE* c.662G>T variant was confirmed by Sanger sequencing as explained above. Results showed the presence of the

mutation in heterozygote status for both the index and the father (Figure 2; ClinVar accession SCV001737568). Another mutation has been found in intronic region (intron 5/10): c.662+99G> T. This variant is benign as it is a very common polymorphism in African populations (VarSome database).

3.2 | Molecular modeling

In many publications, it has been reported that M-D has been linked to all types of variants including single-exonic deletions, interstitial deletions, indels, in-frame deletions, nonsynonymous single-nucleotide missense, and splice, and the vast majority of indels lead to frameshifts and stops. Le Doux²⁵ has compiled all variants reported and ranked them by scores of deleteriousness, within the gnomAD v2 dataset. The new mutation

TABLE 1 Prediction of the effect of the p. Gly221Val missense mutation

Software	Prediction	Score
<i>PolyPhen</i>	Probably damaging	1
<i>SIFT</i>	Damaging	0
<i>Provean</i>	Deleterious	−8.442
<i>Condel</i>	Deleterious	0.624
<i>Mutation Assessor</i>	Medium functional impact	2.16
<i>Mutation Taster</i>	Disease causing	−

c.662G> A has not been reported before, but the same position has been found, causing Gly-to-Asp protein change,²⁶ and was the unique missense mutation found in this cohort among thirteen different pathogenic variants (including nonsense mutations and deletions). This mutation was considered as causing disease since it was present in a three-generation family, cosegregated with the motor disorder, and characterized by a typical autosomal dominant pattern of inheritance with reduced penetrance due to maternal imprinting. Interestingly, another missense mutation (c.662G> T) in the same position was established in this study with familial history of occurrence of the MD in several family members spanning two generations (5 females vs one male). This missense mutation is added to other six missense variants reported in Ref. [25] in which the CADD_PHRD scores range from 23.8 to 35 and are predicted to cause disease by MetaLR, MetaSVM, and MutationTaster. REVEL_rankscores ranged from 0.852 (p. Thr36Arg) to 0.997 (p. Tyr115Cys).

To understand the effect of the new mutation, a molecular modeling has been conducted. The p. Gly221Val substitution was predicted to be damaging according to six bioinformatics programs including PolyPhen, *SIFT*, *Proven*, *Condel*, *Mutation Assessor*, and *Mutation Taster* (Table 1). This mutation was not found in Exome Variant Server (EVS) and Exome Aggregation Consortium (ExAC) databases. Moreover, it seems that the Gly amino acid at this position is very important to have a

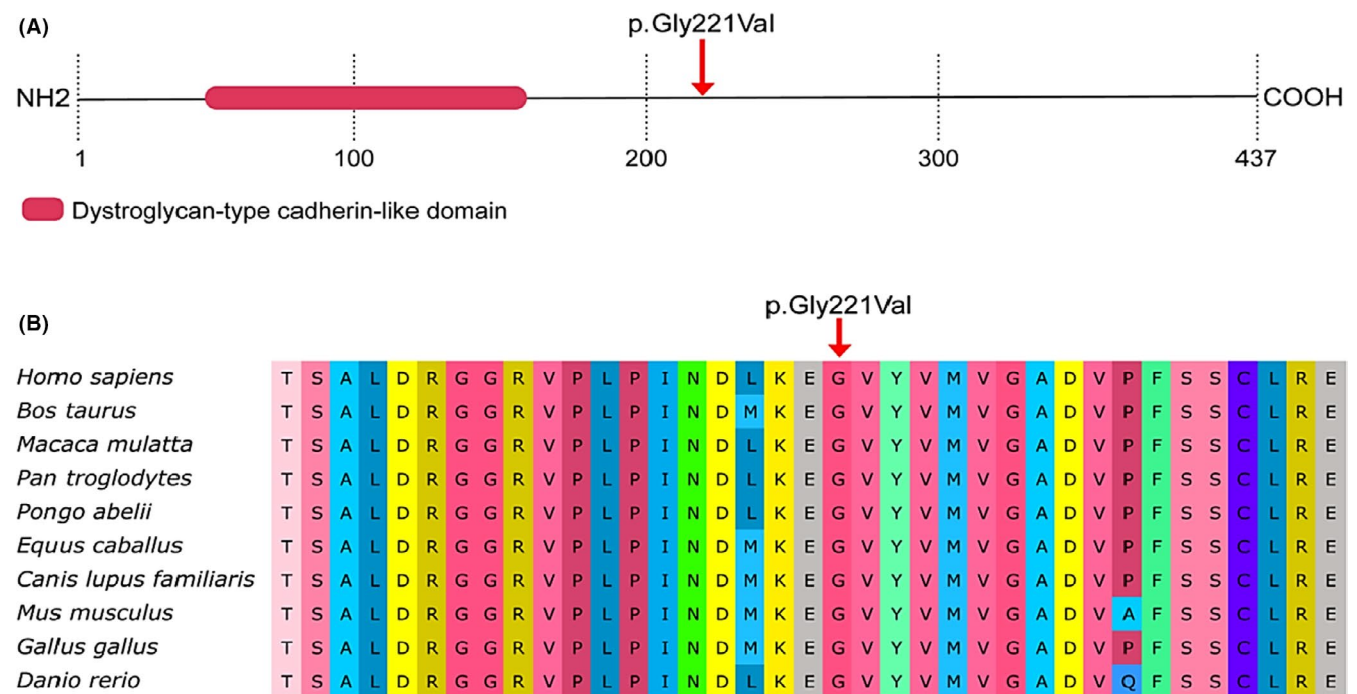


FIGURE 3 Conservation analysis of Gly221 residue. (A) Schematic of SGCE protein. (B) Multialignment of the amino acid sequences of SGCE orthologous proteins

FIGURE 4 Molecular modeling comparison of wild-type and mutant *SGCE* proteins. (A) location of the mutated amino acid in the 3D structure of the *SGCE* protein. (B) The wild-type protein. (C) The mutant protein. Green lines represent hydrophobic interactions

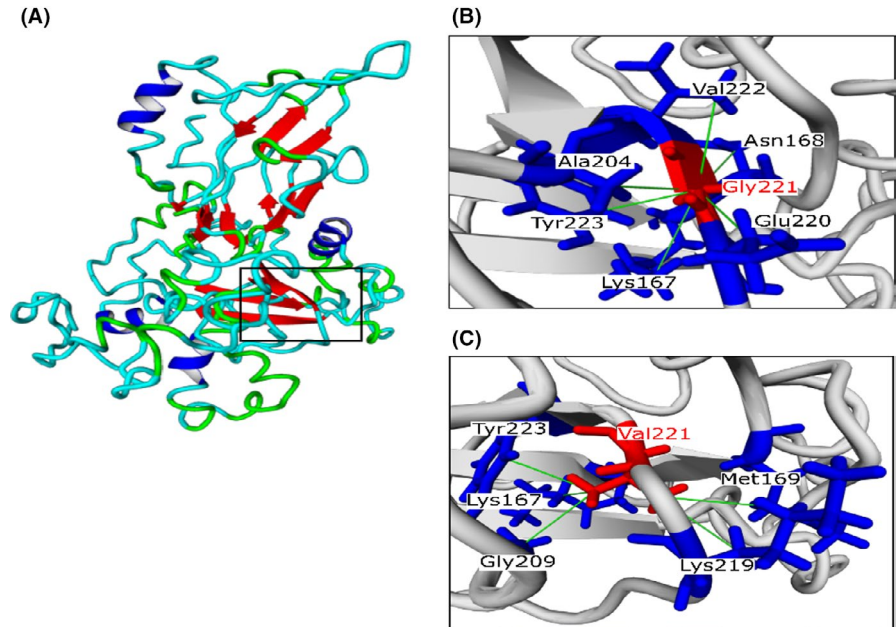


TABLE 2 Prediction of the amino acid change on the protein stability

Software	Prediction	Stability change ($\Delta\Delta G$: Kcal/mol)
mCSM	Destabilizing	-0.509
SDM	Destabilizing	-0.44
DUET	Destabilizing	-0.316
CUPSAT	Destabilizing	-6.46
DeepDDG	Destabilizing	-1.796

normal protein function confirmed by multisequence alignment of the human *SGCE* protein and its orthologous proteins, which showed high conservation of the Gly221 amino acid among species (Figure 3A,B). A 3D structure of *SGCE* protein was built to explore the structural impact of the p. Gly221Val mutation. The amino acid interaction analysis has revealed that this missense mutation may disrupt hydrophobic interactions between the amino acid in position 221 and its adjacent residues. The mutated structure lacks one hydrophobic interaction compared with the native structure. The hydrophobic interactions between Lys167 and Tyr223 residues were conserved in the mutated protein, whereas interactions between Ala204, Glu220, Asn168, and Val222 were replaced by interactions between Gly209, Lys219, and Met169 (Figure 4). In addition, this substitution is likely to decrease the *SGCE* protein stability according to the predictions of CUPSAT, mCSM, SDM, DUET, and DeepDDG programs (Table 2). Thus, the molecular modeling confirmed that mutations, including missense mutation in *SGCE*, translate into either truncated protein or aberrant protein with abnormal function, or degraded

protein with loss of function. This explains the lack of genotype/phenotype correlation.

On the contrary, many studies suggest that age at onset is a strong predictor of the presence of an *SGCE* mutation in case of sporadic M-D. Patients with *SGCE* mutation had a significantly earlier onset of 10 years or less than the noncarriers, which is in accordance with our results. In fact, in familial forms of MD, reduced penetrance and maternal imprinting of the *SGCE* gene cause a “silencing” of the mutated gene within several generations of maternal inheritance, which cannot correlate the phenotype to genotype for male carriers. Also, an intrafamilial variability in clinical symptoms for the same mutation is suggestive of a possible regulatory effect of yet unknown epigenetic or environmental modifiers.

4 | CONCLUSIONS

Herein, we report a first missense mutation inherited in familial pedigree using whole-exome sequencing (WES). Mutations in *SGCE* gene represent the major genetic cause, but other genes and loci are associated with the disease, and in a proportion of patients, no genetic alteration is found. Because of the phenotypic variability of dystonia with the declining costs of next-generation sequencing (NGS), multigene panels, WES, and WGS are very useful for genetic diagnoses of patients with M-D; thus, prenatal testing and preimplantation genetic diagnosis are possible for families in which the pathogenic variant is known.

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None.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

Faiza Chbel: conceptualized the study, analyzed the data, and was responsible for redaction and submission. Hicham Charroute: contributed to molecular modeling and revised the manuscript. Redouane Boulouiz: contributed to molecular analysis and revised the manuscript. Hasna Hamdaoui: performed cytogenetic analysis. Houssein Mossafa: conceived the idea and interpreted the data. Houda Benrahma: analyzed the data and revised the manuscript. Karim Ouldin: contributed to clinical investigation and conception.

CONSENT

I confirm that written patient consent has been signed and collected in accordance with the journal's patient consent policy.

DATA AVAILABILITY STATEMENT

Data openly available in a public repository that issues datasets with DOIs.

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