Detection of a new deleterious SGCE gene variant in Moroccan family with inherited Myoclonic-dystonia

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Abstract

Myoclonus-Dystonia is a neuropsychiatric disorder with autosomal dominant mode of inheritance with variable severity and incomplete penetrance. Pathogenic variants in SGCE are the most frequent genetic cause of M-D with maternal imprinting. Herein we report a new deleterious variant based on protein modeling analysis (c.662G> T) inherited in moroccan family.

Title: Detection of a new deleterious *SGCE* gene variant in Moroccan family with inherited Myoclonic-dystonia

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1. Faiza CHBEL : conception , interpretation, redaction and submission
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Clinical Key Message:

We report the case of a 17-year-old Moroccan girl suffering from myoclonic dystonia showing, using exome sequencing a new c.662G> T variant which is deleterious based on protein modeling analysis. This variant is inherited in this moroccan family.

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 SGCE, are the most frequent known genetic cause of M-D with maternal imprinting and in most cases a symptomatic individual inherits the pathogenic variant from his/her father. Herein we report a missense mutation c.662G> T inherited in M-D moroccan family described for the first time which is deleterious based on protein modeling analysis.

Key words: familial Mycoclonus dystonia, SGCE gene ; Morocco; C.662G>T mutation; modelisation

Main text

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) (Fahn et al., 1998). 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'scramp (Raymond et al, 2003). M-D is compatible with an active life of normal span. Psychiatric disorders have been reported to be associate 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 (<1 year) or later (up to 40 years) (Raymond et al., 2008).

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 it as the 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 close to 95% of children who inherit an *SGCE* pathogenic variant from their mother do not (Grabowski et al.,2002; Müller et al, 2002; Raymond et al., 2008). 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 (Grabowski et al., 2002). In some of these patients a loss of imprinting with subsequent biallelic expression of the SGCE gene has been demonstrated (Müller et al., 2002).

The primary M-D locus identification has been done in a large North American family in 1999 and was mapped on chromosome 7q21.3 (Nygaard et al., 1999) and confirmed in other families (Klein et al., 2000; Asmus et al., 2001; Vidailhet et al., 2001). The SGCE gene consists of 13 exons (exon 1–11, 11b, 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 ε-sarcoglycan gene (*SGCE* , DYT11 locus, MIM 604149) represent the major genetic cause, but not the only one, other loci are associated with the disease like DYT15 locus on chromosome 18p11 and in a proportion of patients no genetic alteration is found (Grimes et al., 2002). Various mutations (>100) in

the*SGCE* gene have been found to cause M-D, including nonsense, missense, deletions and insertions (Asmus et al., 2001). Most of these mutations lead to an abnormally short, non functional ε-sarcoglycan protein that is quickly broken down (Esapa et al., 2007; Misbahuddin et al, 2007). 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 (Raymond, 2012).

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 (Borge et al, 2007, Chen et al, 2008). In Morocco, few studies have been conducted to explore*SGCE* gene mutations responsible of Myoclonus-dystonia (M-D) but a 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 (Rachad et al, 2019). Here, we report the first observation of a familial form of myoclonic dystonia, the case of a Moroccan 17-year-old girl suffering from myoclonic dystonia, using a new approach for medical genetics counselling in National Reference Laboratory (LNR) based on Whole Exome Sequencing (CentoXome Gold®) diagnostic strategy. Molecular explorations in neurogenetics using WES have a positif impact on the patient and his family's medical care management, socio-professional adaptation and genetic counselling and helps to the comprehension of the pathogenicity of mutations.

PATIENTS AND METHODS:

Patients and clinical evaluation:

We report the case of a 17-year-old Moroccan girl suffering from myoclonic 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. 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 ethic committee of Cheikh Khalifa Hospital according to the declaration of Helsinki protocol and all subjects gave a written informed consent before testing.

Methods:

Blood samples were taken from the daughter, father and mother . Blood were either deposited on paper for exome analysis (Centogene) and extracted using Max-QiampDNA extraction kit (Qiagen, Hidden, Germany) according to the instructions of manufacture. DNA was quantified using Qubit (Thermo Fisher Scientific). *SGCE* primers for exon 5 were designed using (Primer 3 software ver 4.1.0) (F: 5'CCTCTGATGAGCCTTGGATT3', and R:(5‘ TTCACAGACCAGGAACTTGAGA3'). PCR were conducted using 100 ng DNA using platinium Hot-start PCR2X masterMix on Veriti thermal cycler (Thermo Fisher Scientific) using cycling conditions (95°C 7 min; 95°C 30s, 58°C 40s 72°C 1 min for35 cycles; 72°C for 15 min) . PCR amplification product was controlled on 2% agarose gel.

For sanger sequencing, the PCR product was purified using exosap method and sequenced using forward and reverse primers using Bigdye Terminator Kit v3.1 cycle sequencing kit (Applied Biosystems, Foster city, CA, USA) and runned on 3500 ABI sequencer (Applied Biosystems, Foster city, CA, USA). Sequence analysis was performed using SeqScape software v2.5 (Ref Seq consensus sequence: NM_003919)

Molecular modelling analysis:

Molecular modelling analysis was performed to predict the effect of the mutation found c.662G>T = p.Gly221Val mutation on the SGCE protein structure. The I-TASSER server (Yang and Zhang., 2015)) 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 Yasara Energy Minimization server (Krieger et al (2009). The change in protein stability upon p.Gly221Val mutation was estimated

using CUPSAT (Parthiban et al., 2006), mCSM (Pires et al., 2014), SDM (Pandurangan et al., 2017), DUET (Pires et al., 2014) and DeepDDG programs (Cao et al., 2019). The YASARA v20.8.23 software (Krieger and Vriend ., 2014) was used for protein structure visualisation.

RESULTS AND DISCUSSION:

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 us 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's a very commun polymorphism in African populations (Varsom database)

Molecular modelling

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, non-synonymous single-nucleotide missense, splice and the vast majority of indels lead to frameshifts and stops. Le Doux, (2020) has compiled all variants reported and ranked them by scores of deleteriousness, within the gnomAD v2 dataset. The new mutation has not been reported before but the same position has been found c.662G> A causing protein change Gly to Asp (Pell et al., 2014). To understand the effect of the new mutation, a molecular modelling 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, multi-sequence alignment of the human SGCE protein and its orthologous proteins showed a highly conservation of the Gly221 amino acid among species (**Figure 3.A and 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 to the native structure. The hydrophobic interactions with Lys167 and Tyr223 residues were conserved in the mutated protein, whereas interactions with Ala204, Glu220, Asn168 and Val222 was replaced by interactions with 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**).

This missense mutation is added to other six missense variants reported in (Le Doux.2020) which the CADD_ PHRED 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). *SGCE* variants predicted to be deleterious by in silico analysis may appear in patients undergoing whole-exome or whole-genome sequencing for seemingly unrelated disorders. it has been estimated that approximately 1/348 individuals in the United States population host a *SGCE* variant with a CADD score [?] 25 (LeDoux, 2020). This is suggesting that M-D and minor phenotypic variants such as mild isolated myoclonus may be underdiagnosed.

CONCLUSION:

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), multi-gene panels, WES

and WGS are very useful for genetic diagnoses for patient 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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REFERENCES:

- Asmus, F., Salih, F., Hjermind, L. E., Ostergaard, K., Munz, M., Kuhn, A. A., Dupont, E., Kupsch, A., Gasser, T (2005) Myoclonus-dystonia due to genomic deletions in the epsilon-sarcoglycan gene. Ann. Neurol. 58: 792-797.
- Asmus, F., Zimprich, A., Naumann, M., Berg, D., Bertram, M., Ceballos-Baumann, A., Pruszak-Seel, R., Kabus, C., Dichgans, M., Fuchs, S., Muller-Myhsok, B., Gasser, T. Inherited myoclonus-dystonia syndrome: narrowing the 7q21-q31 locus in German families. Ann. Neurol. 49: 121-124, 2001.
- Borges V, Aguiar Pde C, Ferraz HB, Ozelius LJ (2007) Novel and de novo mutations of the SGCE gene in Brazilian patients with myoclonus-dystonia. Movement disorders: official journal of the Movement Disorder Society; 22(8): 1208–9.
- Chen XP, Zhang YW, Zhang SS, Chen Q, Burgunder JM, Wu SH, et al. . (2008) A novel mutation of the epsilon-sarcoglycan gene in a Chinese family with myoclonus-dystonia syndrome. Movement disorders: official journal of the Movement Disorder Society; 23(10): 1472–5.
- Deborah Raymond , Rachel Saunders-Pullman, Laurie Ozelius , Margaret P Adam, Holly H Ardinger, Roberta A Pagon, Stephanie E Wallace, Lora JH Bean, Ghayda Mirzaa, Anne Amemiya (2003) SGCE Myoclonus-Dystonia , In: GeneReviews(r) [Internet]. Seattle (WA): University of Washington, Seattle.
- Esapa CT, Waite A, Locke M et al. (2007). SGCE missense mutations that cause myoclonus-dystonia syndrome impair epsilon-sarcoglycan trafficking to the plasma membrane: modulation by ubiquitination and torsina. Hum Mol Genet 16: 327–342.
- Fahn SL, Bressman SB, Marsden CD (1998). Classification of dystonia, Adv Neurol ;78:1-10.
- Grabowski M, Zimprich A, Lorenz-Depiereux B et al. (2002). The epsilon sarcoglycan (SGCE) mutated in myoclonus-dystonia syndrome is maternally imprinted. Eur J Hum Genet 11: 138–144.
- Grimes DA, Han F, Lang AE et al. (2002). A novel locus for inherited myoclonus-dystonia on 18p11. Neurology 59: 1183–1186
- Klein, C., Liu, L., Doheny, D., Kock, N., Muller, B., de Carvalho Aguiar, P., Leung, J., de Leon, D., Bressman, S. B., Silverman, J., et al. (2002) Epsilon-sarcoglycan mutations found in combination with other dystonia gene mutations. Ann. Neurol. 52: 675-679.
- Klein C, Schilling K, Saunders-Pullman RJ et al. (2000). A major locus for myoclonus-dystonia maps to chromosome 7q in eight families. Am J Hum Genet 67: 1314–1319.
- Krieger E, Joo K, Lee J, et al (2009) Improving physical realism, stereochemistry, and side-chain accuracy in homology modeling: Four approaches that performed well in CASP8. Proteins 77 Suppl 9:114–122.
- Krieger E, Vriend G (2014) YASARA View - molecular graphics for all devices - from smartphones to workstations. Bioinforma Oxf Engl 30:2981–2982.
- LeDoux, M. (2020) Population Prevalence of Deleterious SGCE Variants, Tremor and Other Hyperkinetic Movements.; 10(1): 50, pp. 1–9.
- Misbahuddin A, Placzek M, Lennox G, Taanman JW, Warner TT (2007) Myoclonis-dystonia syndrome whith severe depression is caused by exon-skiping mutation in the epsilon sarcoglycan gene. Mov Disord. 15;22(8):1173-5.
- Muller B, Hedrich K, Kock N et al. (2002). Evidence that paternal expression of the epsilon-sarcoglycan gene accounts for reduced penetrance in MD . Am J Hum G 71: 1303-11.
- Nygaard TG, Raymond D, Chen C et al. (1999). Localization of a gene for myoclonus-dystonia to chromosome 7q21- q31. Ann Neurol 46: 794–798.
- Pandurangan AP, Ochoa-Montano B, Ascher DB, Blundell TL (2017) SDM: a server for predicting effects of mutations on protein stability. Nucleic Acids Res 45:W229–W235.

- Parthiban V, Gromiha MM, Schomburg D (2006) CUPSAT: prediction of protein stability upon point mutations. Nucleic Acids Res 34: W239-242.
- Peall KJ, Kurian MA, Wardle M, Waite AJ, Hedderly T, Lin JP, et al. (2014): SGCE and myoclonus dystonia: motor characteristics, diagnostic criteria and clinical predictors of genotype. Journal of neurology, 261(12): 2296–304.
- Pires DEV, Ascher DB, Blundell TL (2014) mCSM: predicting the effects of mutations in proteins using graph-based signatures. Bioinformatics 30:335–342.
- Pires DEV, Ascher DB, Blundell TL (2014) DUET: a server for predicting effects of mutations on protein stability using an integrated computational approach. Nucleic Acids Res 42:W314–W319.
- Rachad L, El Otmani H, Karkar A, ElMoutawakil B, El Kadmiri N , Nadifi S (2019) Screening for SGCE mutations in Moroccan sporadic patients with Myoclonus-Dystonia syndrome, Neurosci Lett, Jun 11;703:1-4.
- Raymond and Ozelius, in Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, Amemiya A, editors. GeneReviews(r)[Internet]. Seattle (WA): University of Washington, Seattle; 2012
- Raymond D, Saunders-Pullman R, de Carvalho Aguiar P et al. (2008). Phenotypic spectrum and sex effects in eleven myoclonus-dystonia families with epsilon-sarcoglycan mutations. Mov Disord 23: 88–592.
- Vidailhet M, Tassin J, Durif F et al. (2001). A major locus for several phenotypes of myoclonus – dystonia on chromosome 7q. Neurology 56: 1213–1216.
- Yang J, Zhang Y (2015) I-TASSER server: new development for protein structure and function predictions. Nucleic Acids Res 43:W174–W181.

TABLES:

Software	**Prediction**	**Score**
Polyphen	Probably damaging	1
SIFT	Damaging	0
Provean	Deleterious	-8.442
Condel	Deleterious	0.624
Mutation Assessor	Medium functional impact	2.16
Mutation Taster	Disease causing	-

Table 1: Prediction of the effect of the p.Gly221Val missense mutation.

Software	**Prediction**	**Σταβιλιτψ ηανγε (ΔΔΓ: Κςαλ/μολ)**
mCSM	Destabilizing	-0.509
SDM	Destabilizing	-0.44
DUET	Destabilizing	-0.316
CUPSAT	Destabilising	-6.46
DeepDDG	Destabilising	-1.796

Table 2: Prediction of the amino acid change on the protein stability

FIGURES LEGENDS:

- **Figure 1:** Pedigree of family with familial myoclonus-dystonia.
- **Figure 2:** Electropherogram showing the *SGCE*c.662G> T mutation (A), in father and daughter and c.662G+99> T
- **Figure 3:** Conservation analysis of Gly221 residue. A: Schematic of SGCE protein; B:Multi-Alignment of the amino acid sequences of SGCE orthologous proteins.

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

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