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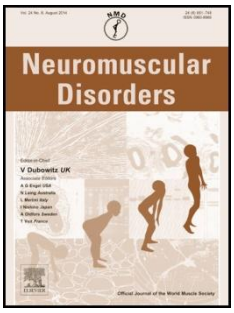

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A new case of Limb Girdle Muscular Dystrophy 2G in a Greek patient, founder effect and review of the literature

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Highlights

- LGMD2G is a rare disorders with only few cases described since now
- We describe a new case of LGMD2G and perform an extensive review of the literature
- We suggest a founder effect for *TCAP* c.90_91del mutation in the Mediterranean area

ABSTRACT

Limb girdle muscular dystrophy (LGMD) type 2G is a rare form of muscle disease, described only in a few patients worldwide, caused by mutations in *TCAP* gene, encoding the protein telethonin. It is characterized by proximal limb muscle weakness associated with distal involvement of lower limbs, starting in the first or second decade of life.

We describe the case of a 37 year old woman of Greek origin, affected by disto-proximal lower limb weakness No cardiac or respiratory involvement was detected. Muscle biopsy showed myopathic changes with type I fibre hypotrophy, cytoplasmic vacuoles, lipid overload, multiple central nuclei and fibre splittings; ultrastructural examination showed metabolic abnormalities. Next generation sequencing analysis detected a homozygous frameshift mutation in the *TCAP* gene (c.90_91del), previously described in one Turkish family. Immunostaining and Western blot analysis showed complete absence of telethonin. Interestingly, Single Nucleotide Polymorphism analysis of the 10 MB genomic region containing the *TCAP* gene showed a shared homozygous haplotype of both the Greek and the Turkish patient, thus suggesting a possible founder effect of *TCAP* gene c.90_91del mutation in this part of the Mediterranean area.

Keywords: Limb Girdle Muscular Dystrophy 2G, Telethonin, *TCAP* gene, founder effect.

1. Introduction

Telethonin (or titin-cap) is a 19 kDa protein, consisting of 167 amino acids, encoded by the *TCAP* gene, which includes two exons and maps on chromosome 17q12 [1]. It is one of the most abundant transcripts in skeletal muscle and localizes to the Z-line of sarcomere in adult skeletal and cardiac muscles [2]. The protein is involved in normal sarcomere assembly and development, as well as in sarcomere-membrane interaction and signalling [1]. It interacts with the titin N-terminus, joining two antiparallel titin (Z1-Z2) domains together, and with other Z-disc proteins, such as LIM protein, Ankyrin Repeat Domain 2 protein, myostatin, potassium channel B subunit minK, protein kinase D, murine double minute 2. In the *TCAP* null mouse and in patients without telethonin expression [3-4], the sarcomere architecture is generally normal. Pathogenesis seems to be related to disruption of sarcomere-T-tubular interaction, as shown in zebrafish models [5].

Mutations in the *TCAP* gene are responsible primarily for Limb Girdle Muscular Dystrophy 2G (OMIM 601954). Until now, only few families affected by LGMD2G have been described [Tab.1A-B]. LGMD2G is an extremely rare disease, originally described in the Brazilian population [4, 6-9] and subsequently reported in patients originating from China [10-11], Moldavia [12], Portugal [13], India [14-16], Spain [17] and Turkey [18].

LGMD2G phenotype is characterised by proximal limb muscles weakness associated with distal involvement of lower limbs with foot-drop. Atrophy generally involves muscles of thighs and the anterior compartment of the legs. Symptoms generally start in the first or second decade of life with lower limbs weakness. The disease is slowly progressive with 40% of patients loosing independent ambulation by the fourth decade. Calf hypertrophy and scapular winging are described in some patients; less frequently, contractures of Achilles tendons can occur. Creatine Kinase (CK) levels are usually increased (3-30x). Cardiac involvement is described only in a few cases [6] and pulmonary function is usually normal. Muscle biopsy shows dystrophic features, sometimes

associated with rimmed vacuoles. At immunostaining for telethonin, both partial reduction or absence of the protein were described.

Mutations in *TCAP* gene can also be responsible for other clinical phenotypes. Recessive homozygous mutations have been reported in a few cases of Congenital Muscular Dystrophy [19-21], and autosomal dominant missense mutations were described in patients with dilated and hypertrophic cardiomyopathy [22]. Patients with LGMD2G skeletal muscle phenotype mainly harbour frameshift or nonsense mutations with an autosomal recessive pattern of inheritance.

2. Case report

A 28 year-old-woman from Thessaly, Greece presented to medical attention for slowly progressive lower limb muscle weakness and atrophy.

Her parents were third grade cousins. No family history of muscular disorders was detected, except for a sister of the grandfather with mild ambulatory difficulties. Birth and psychomotor development were normal; in adolescence, she even practiced martial arts.

Symptoms started at 17 years of age with generalized muscle weakness and difficulties in walking and climbing stairs. Clinical evaluation, performed at age 28, revealed weakness and atrophy of the lower limbs, with major involvement of distal muscles (strength according to MRC scale: gastrocnemius 2/5, tibialis anterior 2+/5, first finger extensor 1/5) in comparison with proximal weakness ileopsoas 4/5 right, 4+/5 left, quadriceps 3+/5 right and 4+/5 left, biceps femoris 4+/5, adductors and abductors of hips 4+/5). There was no involvement of facial, bulbar and upper limb muscles. Tendon reflexes were absent in lower limbs and normal in upper limbs. The patient presented waddling gait along with hyperlordosis and needed a wheelchair to cover longer distances. She was not able to walk on heels and toes.

Muscle MRI of the lower limbs, performed at age 37, demonstrated diffuse and severe fibro-fatty substitution and atrophy of lower limbs muscle associated with a relative sparing of vastus lateralis, sartorius and soleus muscles (Fig.1).

The disease showed a slowly progressive course. At 39 years of age, the patient presented further worsening of proximal lower limb weakness (MRC: tibialis anterior and gastrocnemius 0/5, quadriceps 3/5, iliopsoas 4/5), bilateral foot-drop and need of bilateral support during ambulation; however, the upper limbs were still spared.

Creatine Kinase (CK) levels at onset were increased (2000 U/l) and remained elevated over time at follow-up (407-1200 U/l). Electromyography (EMG) showed myopathic changes. No cardiac or respiratory involvement was detected. Electrocardiogram showed incomplete right bundle branch block, echocardiogram was normal. The patient did not show any cognitive impairment.

Two muscle biopsies were performed at age 22 and 30. Morphological examination was performed according to standard procedures [23]. The first muscle biopsy showed mild myopathic changes along with fibrosis. The second muscle biopsy in the left biceps brachii muscle revealed myopathic changes, type I fibre hypotrophy, multiple central nuclei, fibre splittings, rare cytoplasmic vacuoles, and lipid overload. Connective tissue was normal and no inflammatory infiltrates or necrosis were detected (Fig. 2A). Ultrastructural examination confirmed metabolic abnormalities characterised by a mild increase of intracellular lipids (Fig. 2E). Immunohistochemical analysis showed normal signal for caveolin-3, dysferlin, dystrophin, merosin, α and γ-sarcoglycan. Glycolytic enzymes were normal despite of metabolic alterations shown at electron microscopy.

In order to exclude a possible cause of distal myopathy, the Glucosamine (UDP-N-Acetyl)-2-Epimerase/N-Acetylmannosamine Kinase (*GNE)* gene was analysed with normal results. The sample of the patient was further studied with Next Generation Sequencing (NGS) techniques. DNA samples underwent MotorPlex protocol, analyzing the coding regions of 89 genes; 2x100 bp libraries were run on a HiSeq 1000 instrument (Illumina) and analyzed through a custom pipeline of

bioinformatics analyses [24-25]. We detected predicted pathogenic variants in *MYBPC3* (c.3589A>G; p.Thr1197Ala) and *SYNE2* (c.976G>A; p.Asp326Asn) genes and a homozygous loss-of-function change in *TCAP* (c.90_91del) gene. Considering clinical and histological features of the patient, the most likely pathogenic change among these was in *TCAP*. In fact the clinical phenotypes associated with *MYBPC3* and *SYNE2* mutations, respectively autosomal dominant cardiomyopathy and autosomal dominant Emery-Dreifuss muscular dystrophy phenotype, were not consistent with the clinical presentation of our patient.

The mutation in *TCAP* gene is a homozygous 2 bp deletion in exon 1 which determines the frameshift of the transcript (p.Ser31HisfsX11). In order to confirm the inactivating effect of the mutation we performed immunostaining and Western-blot analysis of telethonin using Rabbit polyclonal anti TCAP antibodies (abCAM, ab121868 - Santa Cruz Biotechnology , Santa Cruz, CA). The analysis revealed complete absence of the protein with both methods (Fig. 2C; Fig 3). Unfortunately parental DNA was not available to confirm the segregation of the mutation, but the consanguinity and functional null effect of the mutation both support the homozygous status.

Interestingly, the same variant has been recently identified [18] in a 35-year-old female patient of Turkish ethnicity with LGMD2G phenotype. In both cases, diagnosis was obtained by NGS approach. We revised the patient´s NGS data for informative markers located 5'and 3' of the *TCAP* gene to investigate the presence of a possible founder effect. We identified a homozygous region extending at 3' for 10Mb including the SGCA gene (Suppl. Fig. 1).

3. Discussion

LGMD2G is a very rare form of Limb Girdle Muscular Dystrophy with autosomal recessive inheritance. Until now, only 27 cases have been described, representing 15 independent patients, since in five families there was more than one affected person in the same generation (Tab.1A-B). The majority of these patients were of Brazilian descent, and the c.157C>T (p.Gln53X - exon 2)

mutation in homozygous state is the most frequent mutation. Although cases are rare world-wide, *TCAP* mutations are likely to occur in every continent.

Here we describe a frameshift mutation in the *TCAP* gene causing a proximo-distal myopathy, which had been detected by next generation sequencing techniques. The clinical phenotype was consistent with LGMD2G, and absence of telethonin at WB analysis confirmed the diagnosis.

Interestingly, the same mutation has been recently described in a 35-year-old Turkish girl with LGMD2G phenotype [18]. Markers reconstruction from NGS data in a 10 Mb genomic distance across the *TCAP* gene demonstrated an identical homozygous haplotype in these two patients, thus suggesting a possible founder effect for this recessive mutation.

The description of our new case further confirms that this mutation is associated with the milder spectrum of the disease, showing onset of weakness in adolescence. So far, the mean age of weakness onset in the literature had been described with 8.5 ± 9.2 years of age (Tab 1), while the two patients with the c.90_91del mutation complained of weakness respectively at the age of 22 and 17 years. This mutation, as well as all of others described so far in other LGMD2G subjects, is a truncating one, while dominant missense mutations are described in cases of cardiomyopathy [19]. The position of the new stop codon created by the mutation seems not be responsible for the phenotype, as well as the entity of protein reduction at Western-blot analysis. Although preliminary, these data argue against a direct correlation between mutation type, residual muscle protein level and clinical phenotype, as far as age at disease onset is concerned. Other modulating factors might be involved in determining part of the clinical variability. Interestingly, tendon contractures and myalgia were not seen in our patient, while these symptoms already had appeared at a very young age (2 years of age) in the patient described by Ikenberg *et al* [18]. Upper extremities were spared in both cases at last evaluation, respectively at 39 and 34 years, which is atypically mild for LGMD2G, since none of the patients previously described had a similar distribution at last evaluation.

On the contrary, distal involvement is frequently described in LGMD2G patients, sometimes also at early stages [6-14-19], especially in lower limbs, presenting with foot-drop and involvement of tibialis anterior muscles. As well as in our patient, this feature was predominant also in other subjects with LGMD2G [6-17]. Respiratory involvement or cardiomyopathy was not reported in both cases, consistently with the majority of previous reports; extrasystolia had been described only in the Turkish patient. CK levels were similar to those detected in other patients (x2-50). Sparing of the sartorius muscle was seen in both muscle MRIs as described previously [12-14; 17].

The muscle biopsy of our patient showed a pattern consistent with other cases of LGMD2G reported in literature, with type I fibre atrophy, central nuclei, fibre splittings and myopathic changes without connective tissue increase and normal sarcomeric structure [4, 9, 13]. We did neither detect lobulated fibres nor classical rimmed vacuoles, as seen in other reports [4, 9, 13]. However, we detected rare cytoplasmic vacuoles and lipid overload, confirmed by ultrastructural examination showing metabolic abnormalities such as mild increase of intracellular lipids (Fig. 2E).

Glycogen deposits and metabolic alterations were an atypical finding, since until now, focal intrasarcoplasmic glycogen deposits were only described once in LGMD2G [9]. This additional finding in our patient enlarges the morphological spectrum of the disease.

4. Conclusion

This case both contributes to consolidate a typical phenotype of LGMD2G and further expands the clinical spectrum of LGMD2G, since it confirms that also milder forms of the disease may exists. Moreover it suggests a founder effect for the *TCAP* c.90_91del mutation. Although telethoninopathy is not a frequent cause of muscular dystrophy - in our previously described LGMD cohort [26] it accounts only to 1% of LGMD patients - it is useful to consider this form of LGMD in the differential diagnosis, especially in patients presenting with distal involvement of the lower

limbs. Interestingly, the muscle biopsy of our patient presented some atypical features, expanding the morphological spectrum of the disease.

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FIGURE LEGENDS

Fig.1 Muscle imaging. Muscle MRI axial T1 images at thigh (A) and calf (B) level demonstrated diffuse and severe fibro-fatty substitution and atrophy associated with relative sparing of vastus lateralis, sartorius and soleus.

Fig.2 Muscle biopsy

Biceps muscle biopsy revealed myopathic signs with type I fibre hypotrophy, size variability, multiple nuclear centralization, fibre splittings rare cytoplasmic vacuoles and lipid overload (A Gomori Trichrome 40x and inhomogeneous enzymatic activity distribution (NADH-TR: B 40x and F. 20x). IHC analysis with Rabbit polyclonal anti TCAP antibodies showed absence of signal in the patient (C) compared to control (D). Ultrastructural analysis showed small lipid droplets in rare muscle fibers (E bar 700 nm; 7000 x)

Fig. 3 Western-blot analysis. Telethonin Western-blot analysis showed protein absence in the patient sample (pt) compared to control (Ctr)

Suppl. Fig.1 Haplotype analysis

The analysis of the informative markers located 5'and 3' of TCAP in our patient (A) and in the patient described by Ikenberg et al. (B), showed a homozygous region extending at 3' for 10Mb including the SGCA gene suggesting a possible founder effect. The variants with the same state of zygosity in both patients are highlighted in green.

Table 1A TCAP mutated cases review of the literature – Clinical Aspects

Family/patient	Clinical presentation										
	Age of onset	Origin	Weakness	C/M	Scapula winging	Retraction	Calf hypertrophy	Ambulation loss	Cardiac Involvement	Respiratory involvement	CK
I/6	9-15 y	Brazil	UL P+D	ND	ND	No	No	4/6 pt (31-39 y)	3/6 pt	No	x3-17
II/3	9-15 y	Brazil	UL P+D; LL P+D	ND	ND	No	No	No	No	No	Elevated
III/3	2-15 y	Brazil	UL P+D; LL P+D	ND	ND	No	Yes	1/3 pt (27 y)	No	No	x10-30
IV/1	8 y	Brazil	UL P; LL P+D	ND	Yes	TT	No	41 y (bone fracture)	No	No	x2
V/1	1 y	Caucasian	LL P+D; Early facial involvement ; UL	No	ND	No	Yes	No (29 y)	No	No	x8
VI-VII/3	1^-2^ decade	China	UL P+D; LL P+D	ND	Yes	ND	Yes	ND	No	No	ND
VIII/2	2^ decade	Chinese-Cambodian	UL P; LL P+D	ND	Yes	TT	Mild	No (32y)	No	No	ND
IX/1	15 y	Moldavia	LL P+D	yes	Mild	TT	++	No (20 y)	No	No	x10
X/1	20 y	Portugal	LL P+D; UL	No	Mild	TT, elbows	Yes	No (50 y)	No	No	x3
XI/1	10 y	India	UL P; LL P	NR	Yes	Yes	Yes	No (10y)	No	No	x15
XII/1	21 y	India	UL P; LL P	NR	No	Yes	No	no (21y)	No	No	x8

XIII/1	8 y	India	LL P+D	ND	Yes	TT, elbows	ND	44 y	No	Yes (FVC 65%)	x50
XV/1	2 y	Spain	LL P+D; UL	ND	Yes	TT, patellar	Yes	No (29 y)	No	No	x2-5
XVI/1	2 y	Turkey	LL P+D	No	No	yes	No	Yes (28y)	No	No	x5
XVII/1	1 y	France	UL P; LL P+D	ND	Yes	TT, hip, lumbar spine	Yes	No (12y)	No		x5
XVIII/1	5y	Brazil	ND	ND	ND	ND	ND	ND	ND	ND	ND

Y: years, UL: upper limbs, LL: lower limbs, C/M cramps/Myalgia; ND: not determined; NR: not reported;

Table 1B TCAP mutated cases review of the literature – Bioptical and molecular aspects

Family / patient	**Muscle biopsy**							**Genetic analysis**	**Reference**
	Fibrosis	**Fibral predominance**	**Rimmed vacuoles**	**Lobulated fibers**	**IHC /WB telethonin**	**Peculiar aspects**	**Ultrastructural analysis**	**TCAP mutations (NM_003673.3)**	
I/6	Yes	No	Yes	NR	Absence	No	ND	c.157C>T (p.Gln53X)/c.109_110delGG (p.Gly37Leufs)	[4,6-7]
II/3	Yes	ND	Rare	NR	Absence	No	ND	c.157C>T (p.Gln53X)	[4,6]
III/3	Yes	ND	No	NR	Absence	No	ND	c.157C>T (p.Gln53X)	[4]
IV/1	Yes	Type I	No	Yes	Absence	No		c.157C>T (p.Gln53X)	[8]
V/1	Yes	Type II	yes	No	Absence (IHC)	Fibrosis, neurogenic-like fiber abnormalitiesfocal intrasarcoplasmic glycogen deposits	Glycogen deposits, autophagic vacuoles	c.157C>T (p.Gln53X)	[9]
VI-VII/3	ND	ND	ND	NR	Absence	No	ND	c.26_33dupAGGTGTCG (p.Glu12argfsX20)	[10]
VIII/2	ND	ND	ND	NR	ND	No	ND	c.26_33dupAGGTGTCG (p.Glu12argfsX20)	[11]
IX/1	Yes	Type I	No	Yes	Absence	No	Preserved structure	c.25G>A (p.Trp25X)	[12]
X/1	Yes	Type I	Yes	Yes	ND	No	ND	c.157C>T (p.Gln53X)	[13]

XI/1	Yes	NR	No	Yes	NR	No	ND	c.32C>A p.(Ser11X)	[14]
XII/1	No	NR	No	Yes	NR	No	ND	c.26_33dupAGGTGTCG (p.Glu12argfsX20)	[14]
XIII/1	No	Type II (atrophy type I)	No	Yes	Reduction (IHC)	No	ND	c.244C>T (p.Gln82X)	[15]
XV/1	No	58% type I	No	Yes	Absence (WB)	No	ND	c.255C>A (p.Tyr85X)	[17]
XVI/1	Yes	Type I	No	Yes	Absence	No	ND	c.90_91del (p.Ser31Hisfs*11)	[18]
XVII/1	No	Type I atrophy	No	No	NR	No	ND	c.172C>T (p.Gln58X)	[19]
XVIII/1	ND	ND	ND	ND	Absence	ND	ND	c.157C>T (p.Gln53X)	[20]

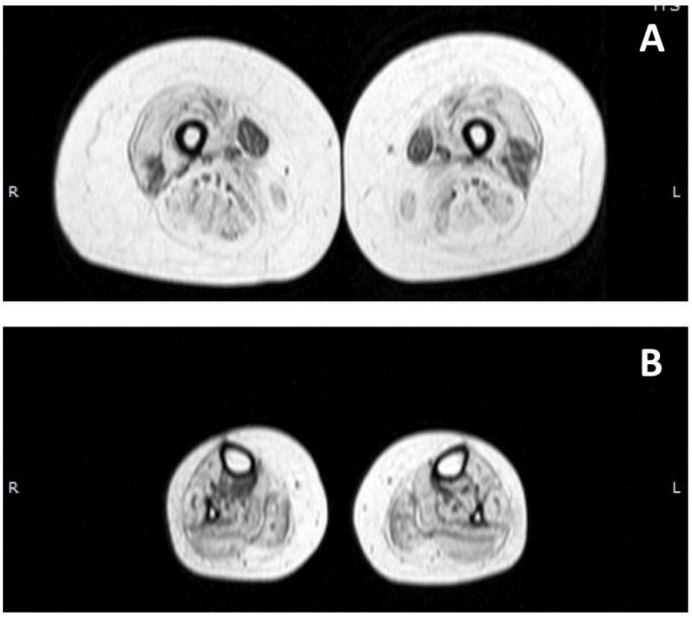

Fig. 1 Muscle MRI.tif

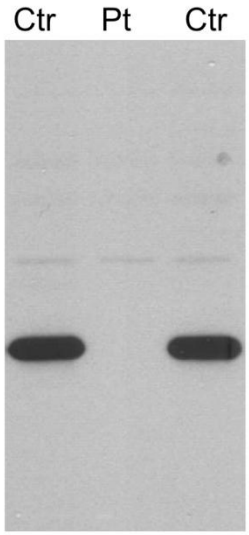

Fig. 3 Western blot.jpg

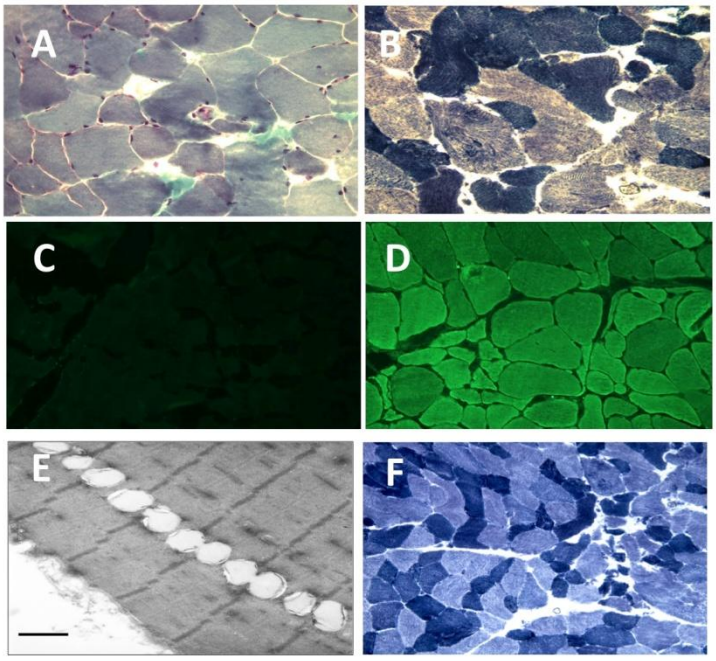

Fig.2 biopsy R January.jpg

Greek patient

A

Chromosome	Position	Ref	Var	Zygosity	Gene	Gene position/function
chr17	10448361	C	A	HET	MYH4S	ncRNA_intronic
chr17	10448769	A	T	HOM	MYH2	exonic/synonymous SNV
chr17	10450816	T	C	HOM	MYH2	exonic/synonymous SNV
chr17	37821700	CTG	C	HOM	TCAP	exonic/frameshiftdeletion
chr17	37822311	A	C	HOM	TCAP	exonic/synonymous SNV
chr17	40556515	T	C	HOM	PTRF	UTR3
chr17	48243504	C	T	HOM	SGCA	intronic
chr17	48245180	C	T	HOM	SGCA	intronic
chr17	48246231	C	T	HOM	SGCA	intronic
chr17	48246827	C	T	HOM	SGCA	intronic
chr17	48252804	T	C	HOM	SGCA	UTR3
chr17	78078709	T	C	HET	GAA	exonic/synonymous SNV
chr17	78079481	C	G	HET	GAA	intronic

Turkish patient

B

Chromosome	Position	Ref	Var	Zygosity	Gene	Gene position/function
chr17	10448361	C	A	HET	MYH4S	ncRNA_intronic
chr17	10448769	A	T	HOM	MYH2	exonic/synonymous SNV
chr17	10450816	T	C	HOM	MYH2	exonic/synonymous SNV
chr17	37821700	CTG	C	HOM	TCAP	exonic/frameshiftdeletion
chr17	37822311	A	C	HOM	TCAP	exonic/synonymous SNV
chr17	48243504	C	T	HOM	SGCA	intronic
chr17	48245180	C	T	HOM	SGCA	intronic
chr17	48252804	T	C	HOM	SGCA	UTR3
chr17	78078709	T	C	HET	GAA	exonic/synonymous SNV
chr17	78079481	C	G	HET	GAA	intronic

Ref: wild-type reference; Var: nucleotide variation

Suppl Fig 1 Jan.jpg