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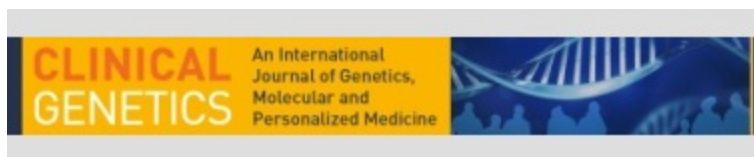
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Novel phenotype in STIM1-related disorders including bones abnormalities and absence of immune defect

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Abstract:	<p>STIM1, the stromal interaction molecule 1, is the key protein for maintaining calcium concentration in the endoplasmic reticulum by triggering the Store Operated Calcium Entry (SOCE). Bi-allelic mutations in STIM1 gene are responsible for a loss of STIM1 function in patients affected with a CRAC channelopathy syndrome in which severe combined immunodeficiency syndrome, autoimmunity, ectodermal dysplasia and muscle hypotonia are combined.</p> <p>Here, we studied two siblings from a consanguineous Syrian family, presenting with muscle weakness, hyperlaxity, elastic skin and tooth abnormalities, but no immune disorders. Patients presented with bones abnormalities such as dysmorphic facies and hypoplastic patellae. Using exome sequencing, we have identified a new homozygous frameshift mutation in STIM1: c.685delT [p.(Phe229Leufs*12)], leading to a complete loss of the full-length STIM1 protein.</p> <p>In this study we describe a new phenotype linked to STIM1 mutations. This is to our knowledge, the first description of the involvement of STIM1 causing bone abnormalities. Moreover, we show that the complete loss of STIM1 function is not always associated with immune disorders. Altogether our results broaden the spectrum of phenotypes associated with mutations in STIM1 and opens new perspectives on the pathological mechanisms associated with a defect in the proteins constituting the SOCE complex.</p>

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Novel phenotype in STIM1-related disorders including bones abnormalities and absence of immune defect

[short title : new phenotype for a STIM1 mutation]

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ABSTRACT

STIM1, the stromal interaction molecule 1, is the key protein for maintaining calcium concentration in the endoplasmic reticulum by triggering the Store Operated Calcium Entry (SOCE). Bi-allelic mutations in *STIM1* gene are responsible for a loss of STIM1 function in patients affected with a CRAC channelopathy syndrome in which severe combined immunodeficiency syndrome, autoimmunity, ectodermal dysplasia and muscle hypotonia are combined.

Here, we studied two siblings from a consanguineous Syrian family, presenting with muscle weakness, hyperlaxity, elastic skin and tooth abnormalities, but no immune disorders. Patients presented with bones abnormalities such as dysmorphic facies and hypoplastic patellae.

Using exome sequencing, we have identified a new homozygous frameshift mutation in *STIM1*: c.685delT [p.(Phe229Leufs*12)], leading to a complete loss of STIM1 protein.

In this study we describe a new phenotype linked to STIM1 mutations. This is to our knowledge, the first description of the involvement of STIM1 causing bone abnormalities. Moreover, we show that the complete loss of STIM1 function is not always associated with immune disorders. Altogether our results broaden the spectrum of phenotypes associated with mutations in *STIM1* and opens new perspectives on the pathological mechanisms associated with a defect in the proteins constituting the SOCE complex.

Keywords: CRAC, STIM1, Muscles, SOCE

INTRODUCTION

Calcium reservoirs are regulated by Ca²⁺ Released Activated Channels (CRAC), part of the Store-Operated Calcium Entry (SOCE). Two proteins form the CRAC channels : Stromal interaction molecule 1 (STIM1)¹ and ORAI1². The activation of the CRAC channels is triggered by STIM1, an endoplasmic reticulum transmembrane protein after calcium depletion.

Mutations in CRAC channel proteins, especially in STIM1, are associated with different diseases depending on the effect of the mutation on protein function³. On one hand, gain of function mutations are associated with Tubular Aggregate Myopathy (TAM) (MIM 160565), Stormorken syndrome (MIM 185070) and York platelet syndrome. On the other hand, loss-of-function mutations are responsible for CRAC channelopathy, combining severe combined immunodeficiency like (SCID-like) syndrome, autoimmunity, ectodermal dysplasia with dental enamel defects and muscular hypotonia.

Here, we describe a new homozygous frameshift mutation, c.685delT (p.Phe229Leufs*12), in the transmembrane domain of *STIM1* in two siblings from a Syrian consanguineous family, affected with a new combination of symptoms without immune disorders.

MATERIAL AND METHODS

Genetic analyses

After informed consent was obtained from individuals included in the study, blood samples were collected, and genomic DNA was extracted from lymphocytes with the use of standard methods. All protocols performed complied with the ethics guidelines of the institutions involved.

Targeted exome sequencing (TES) of 4813 genes was carried out on individual III.19, IV.11, IV.12 and IV.13 from the family, using the Trusight One Sequencing Panel (Illumina, USA). Library preparation, capture, sequencing, image analysis and base calling were performed by the Genomic and Bioinformatics facility (GBiM) from our institute, Marseille Medical Genetics, as previously described⁴. TES data from individuals were analyzed and segregated using VarAFT software⁵. Refining of the obtained list of candidate variants was performed by removing all variants with a frequency above 1% in several frequency datasets Genome Aggregation Database (gnomAD)⁶, the Greater Middle East (GME) Variome⁷, Iranome⁸, and *in-house* database.

The candidate variant in *STIM1* was tested by Sanger sequencing of PCR amplified fragments from family members, for whom DNA was available, using specific primers (available upon request).

Cell culture

Immortalized lymphoblastoid cell line from individuals and controls were prepared at the accredited Biological Resource Center (CRB TAC), Marseille's Timone Hospital.

Reverse Transcriptase PCR (RT-PCR) and quantitative Real-Time PCR (qRT-PCR)

RT-PCR was performed on RNAs extracted from lymphoblastoid cells and qRT-PCR was performed with specific primers targeting *STIM1*, *STIM2* and *ORAI1* genes (available upon request).

Western blot

Western blot analysis was performed on protein extracts from lymphoblastoid cells using anti-STIM1 antibody (Sigma Aldrich, #S6072), anti-STIM2 antibody (Sigma-Aldrich, #S8572) and anti-ORAI antibody (abcam, #ab175040).

RESULTS

Clinical description

The subjects were born to healthy first cousin Syrian parents (Figure 1A). Delivery was normal with no recall of exposure to pre- or perinatal environmental toxins. The three other siblings are healthy. There is a positive familial history with 4 boys deceased in their first year of life.

Male patient IV.12 was seen in consultation at 10 years (Figure 1A). Developmental milestones were delayed, as he started walking without help at 3 years. Since that time, his parents mentioned difficulties to walk, run and stand up. According to the parents, various investigations were performed in their own community in Syria and reported as “normal”. He was enrolled in a normal school and his results were at the average level.

When seen, his OFC was 54.5 cm, his weight 43.2 kg, and his length 134.5 cm. He had walking difficulties and a wide based gait. He had a long face, a midface hypoplasia, a beaked nose, a prominent columella, a smooth philtrum, a thin upper lip, prognathia, high and narrow palate, multiple carries, and yellow teeth. Hyperlaxity and hyperelastic skin were noted as well. Neurological examinations disclosed proximal muscular weakness mainly in the lower limbs; atrophy of the calves’ muscles, hammer toes and indentation at the ankles internal bilateral and at the knees external bilateral; and a positive Gowers. Lumbar spine MRI revealed an atrophy of paraspinal muscles. Skeletal survey revealed the presence of hypoplastic patellae. His 8-year-old younger sister (IV.13) presents the same clinical picture.

Identification of a *STIM1* mutation

To identify the mutation responsible for the disease, we realized TES in five individuals from the family. We assumed homozygosity by descent in this family, searching for shared homozygous variants in the two patients (IV.12&13), which are obligate heterozygous in the father (III.19) and either heterozygous or absent in their unaffected older sister (IV.11). The latter hypothesis led to the identification of 46 homozygous variants with 6 having a frequency below 1% in frequency datasets and 2 are in coding regions of *KCNQ1* and *STIM1* genes. The variant in *STIM1* is a novel frameshift mutation: c.685delT (p.Phe229Leufs*12). Knowing that *STIM1* loss of function mutations have been described in CRAC

channelopathies ³, which share clinical signs with the disease affecting our patients, this deletion in *STIM1* was considered as the candidate pathogenic mutation. Segregation studies confirmed that the new variant is homozygous in affected individuals, heterozygous in parents, and never homozygous in the unaffected sibs (Figure 1B). *STIM1* transcript (NM_003156) comprises 12 exons and encodes a 685 amino acid protein (NP_003147.2) (Figure 1C and D). We suggest that this novel c.685delT (p.Phe229Leufs*12) frameshift mutation in *STIM1* is responsible for a new CRAC channelopathy in our patients.

mRNA levels analysis

Premature termination codons often result in non-sense mediated decay of mRNA ⁹. To test this, we performed qRT-PCR using RNA extracted from patients' and controls' immortalized lymphoblastoid cells using specific primers for *STIM1*, *STIM2* and *ORAI1*.

This revealed decreased *STIM1* mRNA levels in all family members as compared to controls. The decrease is more important in patients, as compared to heterozygous individuals: 60% decrease in homozygous patients versus 20% decrease in the heterozygous carriers (Figure 2A). Unexpectedly, this analysis revealed a 50% decrease of *STIM2* mRNA levels in all family members as compared to controls (Figure 2B) whether they carry heterozygous or homozygous mutations. No changes in *ORAI1* mRNA levels were observed (Figure 2C).

Complete loss of STIM1 protein in patients' cells

At the protein level, we observed a complete loss of *STIM1* protein in lymphocytes cells from patients, and 35% decrease in heterozygous individuals, as compared to control (Figure 3A). *STIM2* and *ORAI1* protein levels were unchanged in all tested individuals (Figure 3B and C).

DISCUSSION

In this study, we identified a new homozygous c.685delT (p.Phe229Leufs*12) frameshift *STIM1* mutation in two patients from a consanguineous family, affected with muscle weakness, dysmorphic facies, hypoplastic patellae and hyper elasticity. This frameshift mutation results in a premature termination codon (PTC). The mutations identified so far in *STIM1* in CRAC channelopathies are 3 missense c.494C>A (p.Pro165Gln), c.1276C>T (p.Arg426Cys) and c.1285C>T (p.Arg429Cys) ¹⁰⁻¹²; a splicing acceptor site, c.970-1G>A ¹³ and one insertion c.381insA (p.Glu128fs*9) ¹⁴.

Here, we demonstrated a complete loss of *STIM1* protein in patients homozygous for the c.685delT mutation. These results associated with the drastic reduction of *STIM1* mRNA levels suggest the activation of the nonsense-mediated mRNA decay system ¹⁵. This reduction of *STIM1* mRNA levels is less important in the heterozygous carriers due to the presence of the mutation in only one allele.

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3 This loss of STIM1, has no impact on STIM2 expression, the other member of the STIM protein family
4 ¹⁶, as we show a variable reduction of STIM2 mRNA levels in all family members but not associated
5 with modification of STIM2 protein levels. Similarly, we did not observe any differences in mRNA or
6 protein levels for ORAI1.
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10 One major point of our research is that this novel mutation has been identified in patients presenting a
11 new phenotype characterized by dysmorphic facies, hypoplastic patella, hyperlaxity, elastic skin, and
12 muscle weakness with positive Gowers sign and paraspinal muscles atrophy. Muscular hypotonia and
13 muscle weakness have been reported in many patients with *STIM1* loss of function mutations ³. Our
14 patients present yellow teeth and multiple carries due to enamel defects, a classical symptoms of CRAC
15 channelopathies ¹⁷.
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20 While the importance of SOCE influx in bone health and disease is known, as reviewed in ¹⁸, *STIM1*
21 mutation leading to skeletal abnormalities have been described in only one family ¹⁹. In this study, we
22 confirmed that *STIM1* mutations are responsible for bones abnormalities manifesting in our patients as
23 hypoplastic patellae and dysmorphic facies.
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27 Also, even if our index case (IV.12) has an history of recurrent respiratory infection, there is no evidence
28 of a severe immune disorder in our patients. To date, complete loss of *STIM1* protein have always been
29 associated with severe immune disorders. Here, we identified the first loss of function mutation found
30 in patients who do not present yet any immune disorders. The complete loss of *STIM1* in patients might
31 be compensated by other mechanisms to avoid a severe immune disorder.
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35 Altogether, these data widen the spectrum of *STIM1* mutation associated phenotypes.
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39 **ACKNOWLEDGEMENTS**

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42 We wish to thank the patients, families, and health professionals whose participation made possible this
43 research.
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47 **COMPETING INTERESTS**

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49 The authors have no conflicts of interest to disclose.
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53 **DATA AVAILABILITY**

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56 The data that supports the findings of this study are available from the corresponding author upon
57 reasonable request. The genomic data are not publicly available due to privacy or ethical restrictions
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For Review Only

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FIGURE LEGENDS

Figure 1

Identification of a novel STIM1 mutation. **A:** Family pedigree. **B:** Electropherograms showing the identified *STIM1* mutation c.685delT (p.Phe229Leufs*12). **C, D:** Schematic representation of *STIM1* gene (NM_003156) and protein (NP_003147). Previously identified mutations in CRAC channelopathy are indicated in purple.

Figure 2

mRNA analysis in patients' immortalized lymphoblastoid cells. qRT-PCR analysis of *STIM1* (**A**), *STIM2* (**B**) and *ORAI1* (**C**) mRNA levels in patients and controls. Relative expression was calculated using the $\Delta\Delta C_t$ method \pm SEM, N=3.

Figure 3

Loss of STIM1 protein in patients' immortalized lymphoblastoid cells. Western blot analysis on immortalized lymphoblastoid cells lysates from control and patients for *STIM1* (**A**), *STIM2* (**B**) and *ORAI1* (**C**) protein. N=3.

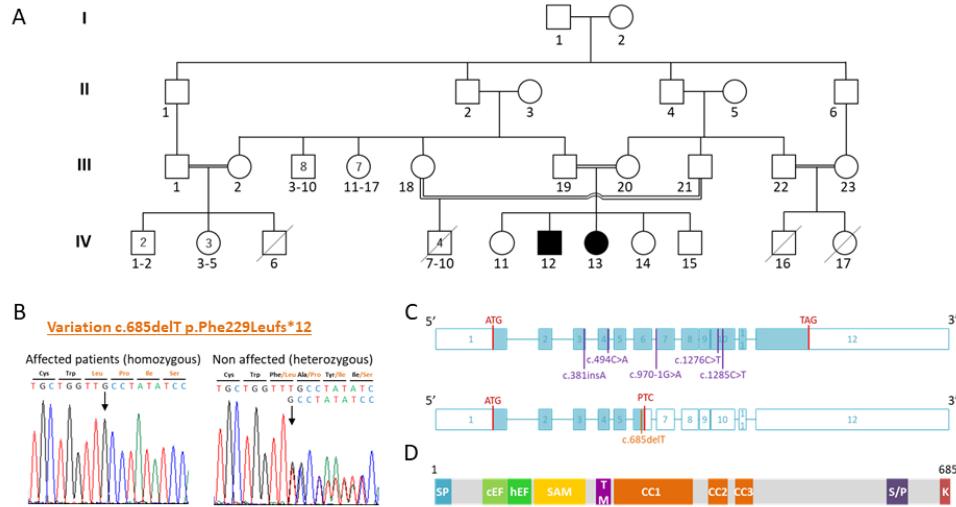


Figure 1

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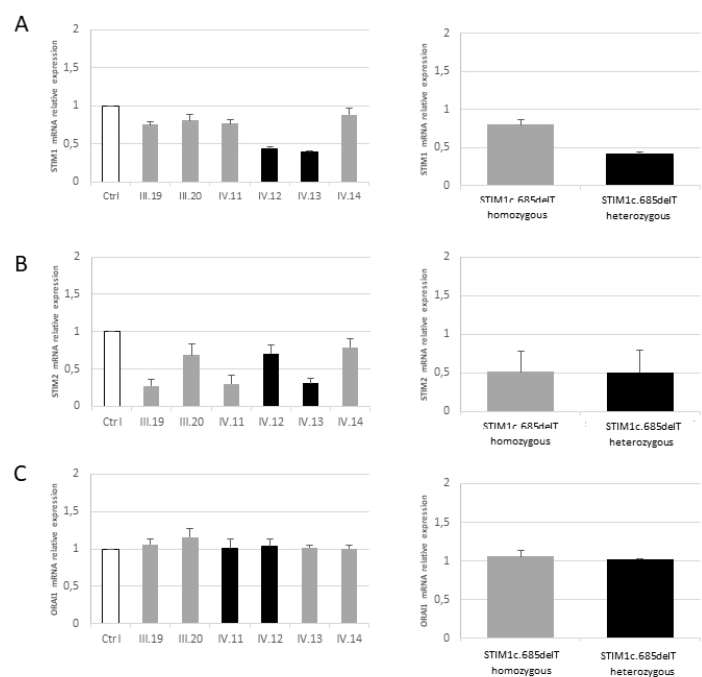


Figure 2

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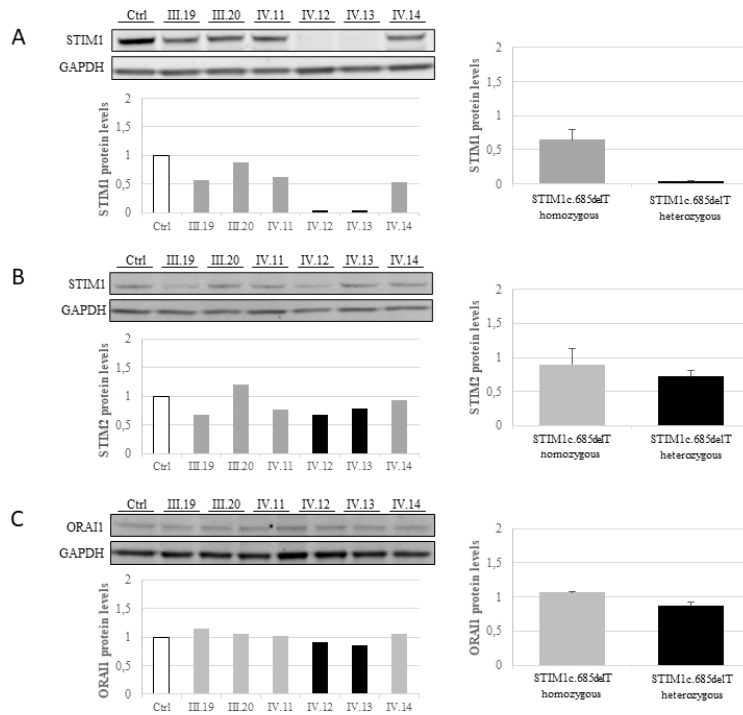


Figure 3

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