

# 1 **Bi-allelic loss-of-function OBSCN variants predispose**

## 2 **individuals to severe recurrent rhabdomyolysis**

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18 **Running title:** *OBSCN* variants underlie rhabdomyolysis  
19

## 1 Abstract

2 Rhabdomyolysis is the acute breakdown of skeletal myofibres in response to an initiating factor, most  
3 commonly toxins and over exertion. A variety of genetic disorders predispose to rhabdomyolysis through  
4 different pathogenic mechanisms, particularly in patients with recurrent episodes. However, most cases  
5 remain without a genetic diagnosis. Here we present six patients who presented with severe and recurrent  
6 rhabdomyolysis, usually with onset in the teenage years; other features included a history of myalgia and  
7 muscle cramps. We identified ten bi-allelic loss-of-function variants in the gene encoding obscurin  
8 (*OBSCN*) predisposing individuals to recurrent rhabdomyolysis. We show reduced expression of *OBSCN*  
9 and loss of obscurin protein in patient muscle. Obscurin is proposed to be involved in SR function and  
10  $\text{Ca}^{2+}$  handling. Patient cultured myoblasts appear more susceptible to starvation as evidenced by a greater  
11 decreased in SR  $\text{Ca}^{2+}$  content compared to control myoblasts. This likely reflects a lower efficiency when  
12 pumping  $\text{Ca}^{2+}$  back into the SR and/or a decrease in  $\text{Ca}^{2+}$  SR storage ability when metabolism is  
13 diminished. *OBSCN* variants have previously been associated with cardiomyopathies. None of the  
14 patients presented with a cardiomyopathy and cardiac examinations were normal in all cases in which  
15 cardiac function was assessed. There was also no history of cardiomyopathy in first degree relatives, in  
16 particular in any of the carrier parents. This cohort is relatively young, thus follow-up studies and the  
17 identification of additional cases with bi-allelic null *OBSCN* variants will further delineate *OBSCN*-  
18 related disease and the clinical course of disease.

19 **Keywords:** rhabdomyolysis; hyperCKaemia; myalgia; exercise intolerance; obscurin

20 **Abbreviations:** AUC: area under the curve; CK: creatine kinase; CTRL: control; MNV: multinucleotide  
21 variant; SR: sarcoplasmic reticulum; UNL: upper normal limit

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# 1 Introduction

2 Rhabdomyolysis is a serious medical condition involving the rapid breakdown of damaged or injured  
3 skeletal myofibres and may require intensive care management. Muscle breakdown results in release of  
4 myofibrillar content into the extracellular space and the circulation, resulting in hyperCKaemia  
5 (hyperCK) and myoglobinuria. Clinically, rhabdomyolysis can range from asymptomatic episodes with  
6 isolated hyperCK to a life-threatening condition with profound myoglobinuria, often progressing to acute  
7 renal failure and requiring intensive care management in severe cases. Clinical features include acute  
8 muscle weakness, myalgia, muscle swelling and elevated CK (defined as >5-times the upper normal  
9 limit).<sup>1</sup> Some patients also develop compartment syndrome necessitating fasciotomy.

10 Rhabdomyolysis can be acquired (trauma, ischaemia, infection and toxin or drug-related)<sup>2</sup> or genetic<sup>3</sup> in  
11 origin. A fascinating history of rhabdomyolysis exists in the literature, with reports dating back to biblical  
12 times and antiquity often in relation to poisoning.<sup>4</sup> Quail poisoning is a well-documented cause of  
13 rhabdomyolysis and is caused by ingestion of toxins associated with quail meat.<sup>4</sup>

14 A schema has been suggested to discern rhabdomyolysis cases with a likely genetic contribution.<sup>5</sup> This  
15 has an acronym of RHABDO (**R**ecurrent episodes, **H**yperCK for a prolonged period, **A**ccustomised  
16 physical activity, **B**lood CK >50xUNL, **D**rug/medication insufficient to explain severity, **O**ther family  
17 members affected/**O**ther symptoms).<sup>5</sup> A large single-centre study showed that the most common triggers of  
18 rhabdomyolysis included ischaemia/anoxia and traumatic muscle injury in non-neuromuscular cases;  
19 whilst in >50% of cases with a known or suspected neuromuscular basis the trigger was exercise.<sup>5</sup>

20 There is a growing list of Mendelian gene defects associated with increased susceptibility to  
21 rhabdomyolysis, including a number of genes involved in muscle metabolism and mitochondrial  
22 function.<sup>3,6</sup> Recessive variants in the lipin-1 gene (*LPINI*) are a common cause of childhood-onset and  
23 severe rhabdomyolysis, sometimes resulting in kidney failure and cardiac arrhythmia.<sup>7,8</sup> Impaired  
24 synthesis of triglycerides and membrane phospholipids have been hypothesised to underlie the  
25 pathogenesis of *LPINI*-mediated rhabdomyolysis.

26 More recently, variants in genes encoding structural muscle proteins have been implicated in  
27 rhabdomyolysis. Bi-allelic variants in the gene encoding muscular LMNA-interacting protein (*MLIP*)  
28 have been shown to underlie a myopathy characterised by mild muscle weakness, myalgia, susceptibility  
29 to rhabdomyolysis and persistently elevated basal CK.<sup>9</sup> Further, Alsaif *et al.* have reported a single case of  
30 rhabdomyolysis associated with a homozygous missense variant in *MYH1*, the gene encoding myosin  
31 heavy chain 2X<sup>10</sup>, and a *MYH1* missense variant is strongly associated with non-exertional  
32 rhabdomyolysis in Quarter horses.<sup>11</sup>

33 In addition, variants in known muscular dystrophy genes can also predispose a patient to rhabdomyolysis;  
34 in some cases rhabdomyolysis can be the presenting symptom of an underlying muscular dystrophy, e.g.  
35 *ANO5*, *CAV3*, *DMD*, *FKRP* and *SGCA*<sup>5,6,12-15</sup> or neurogenerative disease, e.g. *TANGO2*.<sup>5</sup>

36 Variants in *RYR1* encoding the skeletal muscle Ca<sup>2+</sup> release channel (Ryr1) of the sarcoplasmic reticulum  
37 (SR) have been increasingly recognised as an underlying cause of rhabdomyolysis.<sup>16,17</sup> Similarly, likely-  
38 pathogenic variants in genes critical to excitation-contraction coupling and Ca<sup>2+</sup> handling (*CACNA1S* and  
39 *SCN4A*) have been implicated in exertional heat illness and rhabdomyolysis.<sup>5,18</sup> Kruijt *et al.* identified an  
40 underlying genetic diagnosis in 72 of 193 (37%) rhabdomyolysis probands that fulfilled one or more of  
41 the RHABDO criteria, these included variants in 22 disease genes.<sup>5</sup> Despite sequencing of known

1 rhabdomyolysis genes, including via large gene panels and exome sequencing, many individuals who  
2 experience rhabdomyolysis remain without a definitive genetic diagnosis.<sup>5, 19-22</sup>

3 Identification of the genetic cause in a patient with rhabdomyolysis is important because it enables  
4 appropriate advice on how to minimise future episodes, optimised clinical management and genetic  
5 counselling.

6 Obscurin is a component of the sarcomere and localises to the M-band and Z-disks.<sup>23</sup> Obscurin interacts  
7 with titin, myomesin and small ankyrin 1 and is proposed to serve as a linker protein between the  
8 sarcomere and SR.<sup>24-26</sup> Obscurin is also thought to be involved in SR function and Ca<sup>2+</sup> regulation.<sup>27, 28</sup>  
9 *Obscn* null (*Obscn*<sup>-/-</sup>) mice display a mild myopathy, including exercise-induced sarcomeric and  
10 sarcolemmal defects.<sup>27-30</sup> Increased susceptibility of obscurin-deficient muscle to damage may trigger  
11 bouts of rhabdomyolysis in humans.

12 In this study we identified six patients with onset of severe recurrent rhabdomyolysis from 12-27 years of  
13 age and bi-allelic loss-of-function variants in the obscurin gene (*OBSCN*). Four of the six probands  
14 experienced rhabdomyolysis following exercise. Some patients had a history of myalgia and muscle  
15 cramps that preceded the initial episode of rhabdomyolysis. Between episodes, CK levels are normal to  
16 mildly-elevated. We showed reduced *OBSCN* transcript expression and protein abundance in muscle  
17 biopsies from affected individuals. Studies of patient cultured myoblasts showed that starvation condition  
18 induces aberrant Ca<sup>2+</sup> flux into the sarcoplasmic reticulum and higher levels of myoblast death under basal  
19 conditions, the hallmarks of the rhabdomyolysis.<sup>31</sup>

20 Our data clearly demonstrate that bi-allelic loss-of-function *OBSCN* variants predispose individuals to  
21 severe recurrent rhabdomyolysis. *OBSCN* variants should be considered in the diagnosis of patients with  
22 recurrent rhabdomyolysis.

## 23 **Materials and methods**

24 All studies were approved by the Human Research Ethics Committee of the recruiting centre and all  
25 individuals participating in this study gave informed consent. Matching of cases was achieved via  
26 ongoing collaborations and was also facilitated by Metab-L.<sup>32</sup>

## 27 **Clinical investigations**

28 *Patient details and investigations:* We have clinically characterised six probands from six unrelated  
29 families originating from Australia (2), Finland, Turkey, the UK and the USA. Patients presented with  
30 severe rhabdomyolysis, from their teenage years. We performed pedigree analysis, neurological  
31 examination, including muscle strength evaluation according to the Medical Research Council (MRC)  
32 grading scale, serum CK levels during acute episodes and between episodes (baseline), lower limb muscle  
33 MRI and cardiac investigations in some cases, muscle biopsy and genetic workup in the probands and  
34 additional family members. The study was approved by the ethics committees of the participating  
35 institutions. Sample collection was performed after written informed consent from the patients according  
36 to the declaration of Helsinki.

37 *Muscle pathology:* Muscle biopsies were performed in all probands as part of routine diagnostic  
38 investigations. The samples were frozen in liquid nitrogen-chilled isopentane and processed for routine

1 histological and histochemical techniques. Muscle samples were also collected for electron microscopy.  
2 Processing of muscle for light and electron microscopy was performed as outlined previously.<sup>33</sup>

### 3 **Genetic investigations**

4 *AUS1*: DNA from the proband was run on version 1 of a custom designed neuromuscular disease targeted  
5 gene panel at Diagnostic Genomics, PathWest, as detailed in Beecroft *et al.*<sup>22</sup> This did not identify any  
6 likely pathogenic variants. Whole exome sequencing was then performed using the Ion Proton™  
7 (Ampliseq chemistry, Life Technologies). Variant calling was performed using Torrent Suite V3.6.2.  
8 Data were annotated and filtered using an ANNOVAR annotation software suite. Pathogenicity  
9 predictions were made using online prediction software programs: SIFT, PolyPhen-2, and  
10 MutationTaster.

11 *AUS2*: DNA from the proband was sequenced on version 2 the PathWest neuromuscular disease gene  
12 targeted panel.<sup>22</sup> No likely causative variants were identified. DNA was subsequently re-sequenced on  
13 version 5 of the panel, which had been updated to include recently identified skeletal muscle disease  
14 genes, including *OBSCN*. All mapping and calling of variants was done by the BWA Enrichment App  
15 v2.1.2 on the Illumina Basespace Sequence Hub using our custom bed files. Data was analysed in Alissa  
16 Interpret (Agilent).

17 *FINI*: Exome sequencing was performed on DNA from the proband as previously described<sup>34</sup>, and was  
18 analysed as a clinical exome that did not identify pathogenic variants in genes with previous disease  
19 associations in OMIM or ClinVar databases. This patient subsequently underwent targeted resequencing  
20 using the MYOcap gene panel.<sup>35</sup>

21 *TURI*: Exome enrichment was performed using Twist Comprehensive Human Exome  
22 kit according to manufacturer's instructions. Prepared library was sequenced on MGI DNBSEQ-  
23 G400 at 80-100X on-target depth with 150 bp paired-end sequencing at Intergen Genetic  
24 Diagnostic Centre (Ankara, Turkey). Bioinformatics analyses were performed using in-house  
25 developed workflow derived from GATK best practices at Intergen Genetic Diagnostic Centre.

26 *UKI*: Exome sequencing and analysis was performed on the proband's DNA as outlined previously.<sup>36</sup>

27 *USA1*: Clinical exome sequencing was performed on the proband at GeneDX. No known or candidate  
28 pathogenic variants were identified. Re-examination of the exome in light of the association of *OBSCN*  
29 with recurrent rhabdomyolysis, identified a single heterozygous essential splice-site variant in *OBSCN*.  
30 DNA from USA1 was subsequently sequenced on version 3 of the PathWest neuromuscular disease gene  
31 panel. A second nonsense variant was identified in this individual, by the PathWest panel.

32 Bi-directional Sanger sequencing was used to confirm the *OBSCN* variants identified and where familial  
33 DNA samples were available, to examine segregation of the variants.

### 34 **Skeletal muscle RNA-seq**

35 Skeletal muscle RNA-seq data from a cohort of individuals were studied. These individuals included 30  
36 patients with skeletal muscle disease, four patients with isolated hyperCK and one asymptomatic relative  
37 of a skeletal muscle disease proband. We utilised RNA-seq data that were generated using a ribodepletion

1 method, this was to negate potential bias associated with RNA-sequencing of large genes, including  
2 obscurin, in samples generated with a poly-A RNA capture method.<sup>37</sup>

3 RNA was extracted with Qiagen RNeasy Plus Universal Mini Kit (Qiagen, Hilden, Germany) according  
4 to the manufacturer's instructions. The strand specific RNAseq library was prepared using the Illumina  
5 Ribo-Zero Plus rRNA Depletion Kit (Illumina, Palo Alto, CA, USA) at the Oxford Genomics Center,  
6 Wellcome Trust Institute, Oxford, United Kingdom. Sequencing was performed on Novaseq (Illumina),  
7 generating over 80 million 150bp-long reads per sample. Trimmed sequences were mapped against the  
8 hg19 human reference genome using STAR 2.7.0d.

9 To evaluate *OBSCN* exon usage, we analysed pooled junction data from the 35 RNA-seq experiments.

## 10 **Quantitative PCR**

11 RNA was extracted from 30 mg frozen tissue using the RNeasy Fibrous Tissue Mini Kit (Qiagen) as  
12 described by the manufacturer. RNA was quantified with a Nanodrop ND 1000 spectrophotometer  
13 (Thermo Fisher Scientific) and electrophoresed on a 1% agarose gel to confirm RNA integrity and  
14 absence of genomic DNA contamination. The SuperScript III First-Strand Synthesis System (Thermo  
15 Fisher Scientific) was used to synthesize cDNA from up to 1 µg total RNA using random hexamers  
16 according to the manufacturer's protocol. Prior to qPCR, all cDNA's were diluted to the equivalent  
17 starting input of 100 ng RNA with UltraPure water (Thermo Fisher Scientific). The Rotor-Gene SYBR  
18 Green PCR Kit (Qiagen) was used to set up 10 µL reactions containing 1 µL diluted cDNA and 0.8 µM  
19 each of forward and reverse primers (*OBSCN*, *RYR1*, *ACTA1*, *MYOG*, *TBP*, *EEF2*; Supplementary Table  
20 1). Primers were designed to amplify transcript variants 1 (NM\_052843.4, isoform A), 2  
21 (NM\_001098623.2, isoform B), 3 (NM\_001386125.1) and IC (NM\_001271223.2, inferred complete  
22 isoform). There were no predicted off-target products. There are two long non-coding RNA genes that  
23 overlap *OBSCN* and the primers do not amplify these. Primer efficiency (in the range 0.9 – 1.1) was  
24 validated by standard curve. Thermal cycling was performed on the Rotor-Gene Q real-time PCR cyclor  
25 and data were analyzed with the associated software (Qiagen) using a cycle threshold of 0.03. Data were  
26 normalised to the geometric mean of two endogenous control genes (*TBP*, *EEF2*) using the delta-Ct  
27 method. Graphed data represent the mean ± SEM and were generated using GraphPad Prism (V6.02).

## 28 **Western blotting**

29 Frozen muscle biopsies were homogenised in modified Laemmli sample buffer as described previously.<sup>38</sup>  
30 Samples were run in Bio-Rad Criterion 3–8 % tris-acetate gradient gels (Bio-Rad Laboratories, CA,  
31 USA) at room temperature, 100 V, for 4 hours. The proteins were transferred from gels onto PVDF  
32 membranes with a Bio-Rad TransBlot Turbo device (program Standard SD, 60 min), using discontinuous  
33 buffer system, gel/anode buffer 1X CAPS with 0.1% SDS; PVDF/cathode buffer 1X CAPS. Subsequently,  
34 the post-blotting gels were stained with Coomassie Brilliant Blue, and the PVDF membranes were  
35 incubated in primary antibody solution, rabbit anti-obscurin ob59 (1:800 dilution) overnight at 8 °C (anti-  
36 obscurin domain 59 antibody is a gift from Prof. Mathias Gautel). Membranes were incubated with HRP-  
37 conjugated secondary antibody and the bands were detected using ECL (SuperSignal West Femto,  
38 Thermo Fisher Scientific) and ChemiDoc MP digital imager (Bio-Rad). Coomassie-stained gels were used  
39 to visualise titin and nebulin bands which served as size markers and loading controls.

## 1 Cell-based assays

2 *Immunofluorescence microscopy*: Primary myoblasts were fixed and stained as previously described<sup>39</sup>  
 3 with anti-calnexin primary antibody (clone AF18, refsc-23954, Santa Cruz Biotechnology). Images were  
 4 acquired on a confocal Leica LSM700 microscope, equipped with a 63X and a 1.3 numerical aperture  
 5 (NA) oil immersion objective. Quantification and morphological analysis of endoplasmic reticulum was  
 6 done with Icy v1.9.5.1 (BioImage Analysis Unit, Institut Pasteur, France).

7 *Ca<sup>2+</sup> measurements*: Thapsigargin-induced responses were monitored in a FDSS $\mu$ Cell microplate reader  
 8 (Hamamatsu Photonics, Japan) to assess sarcoplasmic reticulum (SR) Ca<sup>2+</sup> content. Myoblasts were plated  
 9 in 96-well plates at a density of 15,000 cells per well and incubated for 1 hour in EBSS or in complete  
 10 HAMF10 medium. Myoblasts were loaded with 4  $\mu$ M Cal-520-AM (AAT Bioquest, CA, USA) for 45  
 11 minutes then washed in recording medium containing (mM) NaCl 116, KCl 5.6, MgCl<sub>2</sub> 1.2, HEPES 20  
 12 (pH 7.3) and 150  $\mu$ M EGTA. Thapsigargin (1  $\mu$ M) was simultaneously added in the absence of external  
 13 Ca<sup>2+</sup> in all wells. Recordings were performed at 37 °C, frequency acquisition was 1 Hz and fluorescence  
 14 signals (F) calibrated by adding 50  $\mu$ M digitonin containing 6 mM Ca<sup>2+</sup> (final concentrations) to obtain  
 15 maximal fluorescence signals (F<sub>max</sub>). Data were expressed as F/F<sub>max</sub> and SR Ca<sup>2+</sup> content areas of the TG-  
 16 evoked responses calculated using Origin software (OriginLab Corp, MA, USA). Thapsigargin was  
 17 purchased from Alomone Labs (Israel) and all other reagents were from Sigma-Aldrich (France).

18 *Apoptosis measurements*: Myoblasts were plated in 96-well plates at a density of 5,000 myoblasts per  
 19 well. Caspase 3/7 green apoptosis assay reagent and NuncLight red reagent for nuclear labelling (ref  
 20 C10423 and 4717, Essen Biosciences Ltd, UK) were added in each individual well at a 1/1,000 final  
 21 dilution. Four phase images and four fluorescent images per well (ex 440-480nm; em 504-544nm and ex  
 22 655nm; em 681nm) were taken using IncuCyte® S3 Life Cell Analysis System (Essen Biosciences Ltd,  
 23 UK). Single green events representing caspase 3/7 positive myoblasts were counted and myoblast number  
 24 was assessed from nuclear counts.

25 *Statistical analysis*: Statistical analysis was performed with GraphPad Prism software using a Mann-  
 26 Whitney test for all image analysis experiments, Ca<sup>2+</sup> and apoptosis measurements.

## 27 Data availability

28 The data that support the findings of this study are available from the corresponding author, upon  
 29 request.

## 30 Results

### 31 Clinical findings

32 Here we report six isolated patients presenting with a clinical picture of severe, recurrent rhabdomyolysis.  
 33 Age-of-onset ranged from 12-27 years of age (median age: 17 years). There is no family history of  
 34 myalgia or rhabdomyolysis in any of the families reported in this study. Three of patients were/are elite-  
 35 level athletes in their chosen fields: FIN1 was a competitive swimmer at a national level, USA1 is an elite



1 high school lacrosse player and UK1 in early adult life competed nationally in the 200m and 400m  
2 distance races without symptoms. Detailed clinical summaries and investigations are available in a  
3 Supplementary document.

4 The triggers in two cases were exercise and heat (AUS1, TUR1), in two only exercise (AUS2, FIN1), in  
5 another both episodes occurred following travel (long-haul flight and bus trips; USA1) and in one the  
6 episodes occurred spontaneously (UK1). All patients experienced recurrent bouts of rhabdomyolysis  
7 (>two events), with one patient experiencing up to six episodes per year. Peak CK levels ranged from  
8 17,000-603,000 IU/L (median 312,500 U/L). Basal CK ranged between normal to mildly elevated  
9 (<1,000 IU/L).

10 Other myopathic features are present between episodes of rhabdomyolysis, including myalgia (5/6),  
11 exercise intolerance (3/6) and muscle weakness (1/6). Three of the six probands experienced acute renal  
12 failure (AUS1, TUR1, USA1), in at least one case necessitating kidney dialysis. In two probands,  
13 rhabdomyolysis was associated with compartment syndrome (AUS1, USA1). In AUS1, compartment  
14 syndrome involved both lower limbs and required fasciotomy; in USA1 compartment syndrome during  
15 two episodes required fasciotomy.

16 None of the cases presented with cardiac involvement or have developed any symptoms of cardiac  
17 disease. There is no history of cardiomyopathy in first degree relatives of the probands.

18 AUS1 had resting tachycardia post presentation with rhabdomyolysis but no clear cardiac involvement.  
19 He had a normal cardiac MRI and normal echocardiogram. Cardiac investigations were also normal in  
20 UK1.

21 The clinical findings in each of the cases are presented in Table 1 and in the Supplementary clinical  
22 summaries. Lower limb muscle MRI in patients AUS1 and FIN1 (Supplementary Figure 1) were normal.

23 Muscle biopsies were available for review in all cases. The findings on muscle biopsies ranged from  
24 within normal limits to non-specific mild myopathic changes and prominent central cores (Figure 2). Mild  
25 subsarcolemmal accumulations of glycogen (FIN1 and USA1), dilated SR and t-tubules (FIN1), mild  
26 increase in internal lipid droplets (TUR1), increased variation in myofibre size (AUS1, AUS2, TUR1,  
27 UK1), internal nuclei (AUS1, TUR1) and central cores in type I myofibres (AUS2).

## 28 Genetics

29 We identified ten rare or novel bi-allelic loss-of-function variants in *OBSCN* co-segregating with  
30 predisposition to rhabdomyolysis in six families (Table 2; Figure1). In *AUS1* we identified a homozygous  
31 nonsense variant in *OBSCN* (NM\_001271223.2 corresponds to the inferred complete (IC) obscurin  
32 isoform; exon 62, c.16230C>A, p.(Cys5410\*)). The variant (*rs1322344930*) is present on four of 273,940  
33 alleles in gnomAD. The variant was confirmed by bi-directional Sanger sequencing; studies of familial  
34 DNA showed that both healthy parents and younger brother were all carriers of the variant. In AUS2 we  
35 identified two variants, a nonsense variant in exon 21 (c.6102G>A, p.(Trp2034\*)) and an essential splice  
36 donor site variant (exon 24, c.7078+1G>T). The nonsense and essential splice-site variants were  
37 maternally and paternally inherited, respectively. One of the unaffected sisters (II:4) also harbours bi-  
38 allelic *OBSCN* variants. We did not have access to RNA-seq data from AUS2, however, we have data  
39 from an unrelated patient that also carries the c.7078+1G>T variant. RNA-seq studies on skeletal muscle  
40 found that the main consequence of the c.7078+1G>T variant is skipping of the first two nucleotides  
41 of exon 25 (data not shown). Thus, the major consequence of this change is likely a frameshift. Both were

1 rare in gnomAD (allele frequency <0.0002), with no homozygotes present. Patient FIN1 harboured bi-  
 2 allelic *OBSCN* deletions; exon36: c.9563\_9576del, p.(Leu3188Argfs\*40) and exon105:  
 3 c.23385\_23386del, p.(Ser7796\*). The c.9563\_9576del variant is novel, whilst the c.23385\_23386del is  
 4 present on 740 alleles in gnomAD including four homozygote individuals. Three of the four homozygotes  
 5 are of Finnish background and the allele frequency in Finns is 0.006. In TUR1 a homozygous rare  
 6 nonsense variant was identified in exon 46 (c.14818C>T, p.(Arg4940\*)). This variant was present on five  
 7 of 209,424 alleles in gnomAD, there were no homozygotes. Sanger sequencing found that each parent  
 8 carried the variant and that her unaffected brother did not carry the variant. The variant was absent from  
 9 ~2,500 Turkish exomes suggesting that this is not a common variant in the Turkish population. UK1  
 10 harboured bi-allelic nonsense variants (exon 31: c.8253G>A, p.(Trp2751\*) and exon 42: c.11122A>T,  
 11 p.(Lys3708\*)). Two healthy siblings had single mono-allelic variants. The c.8253G>A variant is novel  
 12 and the c.11122A>T variant is present on two alleles in gnomAD. USA1 harboured a multinucleotide  
 13 variant (MNV) in exon 2 which is annotated as c.386T>A, p.Phe129Tyr (*rs749567826*) and c.387C>A  
 14 (*rs769050588*), p.Phe129Leu but since they alter the same codon the consequence of these variants is  
 15 c.386\_387delinsAA, p.(Phe129\*). This MNV is present on 114 alleles in gnomAD, including two  
 16 homozygotes. Both homozygotes are within the Ashkenazi Jewish population and the allele frequency in  
 17 this population is 0.012. The second variant in USA1 occurs at the essential splice donor site of exon 90,  
 18 c.21532+1G>A; this rare variant is present on three alleles in gnomAD. The exon 90 splice-site variant is  
 19 inherited maternally and is predicted to result in skipping of exon 90 and a frameshift deletion.

## 20 **Obscurin exon usage**

21 In skeletal muscle, *OBSCN* encodes two canonical isoforms (A and B); obscurin A (~720kD) includes 65  
 22 immunoglobulin domains and two fibronectin III domains along with a number of C-terminal signalling  
 23 domains.<sup>40</sup> The larger isoform, obscurin B (~870kD), has a similar structure to obscurin A but diverges at  
 24 the C-terminal region where it contains two Ser/Thr kinase domains.<sup>40</sup> Exon 21 and 105 are annotated in  
 25 the IC obscurin isoform, however exon 21 is not present in obscurin A or B and exon 105 is only present  
 26 in the long isoform (isoform B). To investigate this, we examined exon usage in RNA-seq data from  
 27 human adult skeletal muscle samples. Exon 21 was present in 93% (80,165 reads supporting the junction  
 28 between exons 20 and 21) of all transcripts (Figure 3A). The shorter isoform of obscurin (obscurin A)  
 29 utilises an alternative 3'UTR in exon 92. RNA-seq analysis showed that 61% of all transcripts correspond  
 30 to this short isoform (90,070 reads supporting the inclusion of the exon 92 alternative 3'UTR). There  
 31 were 39% of reads (*n*=58,551) supporting the skipping of the alternative 3'UTR in exon 92, and  
 32 accounting for the longer isoform (obscurin B; Figure 3A).

33 Similarly, at a protein level, the ratio between obscurin A and B isoforms is almost 50-50 in the adult  
 34 muscles (Figure 3C). We therefore conclude, based on knowledge of *OBSCN* expression, that each of the  
 35 variants identified is predicted to result in loss of a considerable portion of obscurin A and/or B isoforms.

## 36 **Obscurin transcript and protein levels are reduced in patient** 37 **skeletal muscle**

38 To determine whether the homozygous AUS1 variant (c.16230C>A, p.(Cys5410\*)) was associated with a  
 39 decrease in *OBSCN* transcript abundance, we performed qPCR using cDNA obtained from patient muscle

1 biopsy and five unrelated control muscle biopsies. The average normalised *OBSCN* transcript abundance  
 2 of control samples was 11.2-fold greater than in the patient muscle (average normalised *OBSCN*  
 3 transcript abundance of  $4.25 \pm 1.9$  in the control, versus 0.38 in the patient; Figure 3B). To ensure that  
 4 this difference was specific to *OBSCN* transcript, and not an artifact, we also measured the transcript  
 5 abundance of three additional genes; ryanodine receptor 1 (*RYR1*), skeletal muscle alpha-actin (*ACTA1*)  
 6 and myogenin (*MYOG*). For each of these three genes, the transcript abundance measured in the patient  
 7 sample was within the range of values obtained for the control samples (Figure 3B). This indicates that  
 8 the decreased *OBSCN* transcript abundance is likely a real finding.

9 Western blot performed for obscurin in skeletal muscle from FIN1 and AUS1 showed greatly reduced  
 10 levels of both isoforms of obscurin (A and B) compared to three healthy control muscle samples (Figure  
 11 3C). In FIN1 there is some retention of obscurin A, this most probably represents obscurin A arising from  
 12 the allele harbouring the nonsense variant in exon 105 that is excluded in the short isoform A. This  
 13 suggests that the disease manifests due to reduced levels or absence of obscurin protein in patient skeletal  
 14 muscle. Total loading of muscle protein is indicated by band intensities for other large muscle proteins  
 15 (titin and nebulin).

## 16 **Ca<sup>2+</sup> handling is impaired in cultured patient cells**

17 Ca<sup>2+</sup> is tightly regulated in skeletal muscle and impairment in Ca<sup>2+</sup> channel function is a recognised  
 18 mechanism in rhabdomyolysis. Moreover, obscurin is also thought to be involved in SR function and Ca<sup>2+</sup>  
 19 regulation.<sup>27,28</sup> To analyse the SR network, we performed immunolabeling with the antibody anti-calnexin  
 20 on primary myoblasts from a healthy control and patient UK1, in growth medium. By confocal  
 21 microscopy, we did not find any differences in patient myoblasts compared to the control (Figure 4A), not  
 22 even after quantification of myoblast total SR nor in other morphological parameters (sphericity, length  
 23 (Figure 4B), roundness, elongation and flatness (not shown)).

24 We studied regulation of SR Ca<sup>2+</sup> content in myoblasts and found that starvation (EBSS medium) induced  
 25 a decrease in Ca<sup>2+</sup> SR content when compared to normal growth conditions (Figure 4C,  $P < 0.001$ )  
 26 probably reflecting lower efficiency when pumping Ca<sup>2+</sup> back into the SR or a decrease in Ca<sup>2+</sup> SR storage  
 27 ability when cell metabolism is diminished. Susceptibility to starvation is exacerbated in UK1 myoblasts  
 28 as we observed a  $69 \pm 6\%$  decrease in Ca<sup>2+</sup> SR content compared to  $33 \pm 2\%$  in control myoblasts (Figure  
 29 4D,  $P < 0.001$ ). This result suggests that patient myoblasts have a decreased ability to fill the SR during  
 30 starvation conditions for the same reasons as described above. Moreover, obscurin deficiency is  
 31 associated with greater myoblast death under basal conditions as attested by caspase expression (Figure  
 32 4E,  $P < 0.001$ ). When quantified by flow cytometry, cell death in patient myoblasts was 54% compared to  
 33 34% in control myoblasts (Figure 4F). These data are preliminary and further work is required to confirm  
 34 these initial findings.

## 35 **Discussion**

36 Herein we described six patients with susceptibility to severe, recurrent rhabdomyolysis (peak CKs  
 37 ranged from 17,000-603,000 IU/L) due to bi-allelic loss-of-function variants in *OBSCN*. All cases had  
 38 experienced at least two episodes of rhabdomyolysis, with one individual (UK1) experiencing up to 6  
 39 episodes per year. Triggers included exercise (including mild exercise,  $n=4$ ) and heat ( $n=2$ ); in two  
 40 individuals the episodes occur without obvious triggers.

1 In most cases there was a prior history of myalgia and muscle cramps. Basal CK between episodes ranged  
 2 from normal to mildly elevated (<1,000 IU/L). Three patients were/are elite level athletes in their chosen  
 3 fields. Elite athletic performance preceding the onset of neuromuscular disease has been noted  
 4 anecdotally for other genetic neuromuscular diseases; most notably in patients with pathogenic variants in  
 5 *ANO5*, *CAPN3*, *CAV3*, *DYSF* and *RYR1*.<sup>3, 14, 41-44</sup>

6 One sibling (II:4) in Family AUS2 also harbours bi-allelic *OBSCN* variants but has not had  
 7 rhabdomyolysis. She is awaiting formal assessment by a neurologist. This is in keeping with  
 8 rhabdomyolysis requiring an underlying genetic factor in combination with environmental triggers.  
 9 Individual II:4 is not active, as the proband is, and thus it is likely that her levels of physical activity have  
 10 been insufficient to trigger a rhabdomyolysis event. There may also be other factors contributing to  
 11 rhabdomyolysis, for example rhabdomyolysis is recognised to occur more frequently in men. There are  
 12 many individuals in gnomAD that carry well established pathogenic variants underlying exertional  
 13 rhabdomyolysis, hyper CK and malignant hyperthermia. These include individuals homozygous for the  
 14 most common *CPT2* (p.Ser116Leu,  $n=4$ ) and *ANO5* (p.Asn64Lysfs\*15,  $n=2$ ) variants and individuals  
 15 heterozygous for known pathogenic dominantly-inherited *RYR1* variants ( $n=3-30$  per variant). Together,  
 16 these data suggest that bi-allelic loss-of-function *OBSCN* variants are in some instances insufficient on  
 17 their own to precipitate rhabdomyolysis.  
 18 Features on muscle biopsy ranged from unremarkable and minimal non-specific changes through to  
 19 striking central cores in type I myofibres. Increased internal nuclei, mild variation in myofibre size and  
 20 glycogen accumulations were each reported in more than one case. Other features included increased lipid  
 21 droplets, core-like regions, type II myofibre predominance and occasional myofibre atrophy and nuclear  
 22 clumps. Cores have been observed in patients with rhabdomyolysis and likely pathogenic variants in  
 23 genes encoding components of the  $Ca^{2+}$  signalling pathway (*RYR1* and *CACNAIS*).<sup>3, 45</sup> Aberrant  $Ca^{2+}$   
 24 handling was observed in UK1 myoblasts, thus altered  $Ca^{2+}$  signalling may represented a unifying  
 25 mechanism in the development of cores and rhabdomyolysis in patients with *RYR1*, *CACNAIS* and  
 26 *OBSCN* variants.

27 Population variant frequency data shows that all LOF variants for which there are homozygous  
 28 individuals in gnomAD are flagged as a MNV (which together do not result in LOF changes at the amino  
 29 acid level) or have LOF curation notes of “uncertain” or “not LOF” (Supplementary Figure 2). This  
 30 suggests that bi-allelic LOF variants in *OBSCN* are likely to be pathogenic. This association was perhaps  
 31 delayed due to the presence of a relatively common spurious LOF variant in *OBSCN* (p.Arg3252\*  
 32 [AGA>TGA], rs3795786, allele frequency: 0.03, gnomAD: >900 homozygote individuals). However,  
 33 this variant was subsequently annotated as a MNV associated with a protein change of p.Arg3252Leu  
 34 (AGA>TTA). One of the variants (p.(Ser7796\*)) we identified in exon 105 is present in four individuals  
 35 in the homozygous state in gnomAD and is present on one allele in FIN1. This annotation of “uncertain”  
 36 is likely due to this exon not being predicted to cause a null allele in *OBSCN* isoform A. However, this  
 37 exon is expressed at similar levels to flanking exons that are present in the long isoform. Furthermore, by  
 38 western blot we have shown a reduction of *OBSCN* isoform A and B in FIN1 skeletal muscle.

39 Two variants reported in this cohort are present in ~1% of individuals in specific control populations. The  
 40 MNV (c.386\_387delinsAA, p.(Phe129\*)) identified in USA1 is present at an allele frequency of 0.012 in  
 41 individuals of Ashkenazi Jewish ancestry (0.0007 in the total population) and there are two homozygous  
 42 Ashkenazi Jewish individuals in gnomAD. The p.(Ser7796\*) variant present in FIN1 is present at an  
 43 allele frequency of 0.009 in the Finnish population in gnomAD and 0.0038 in the total population. Thus,  
 44 these variants may predispose individuals within these populations to rhabdomyolysis.

45 The finding of similar expression of exons not thought to be included in ‘canonical’ functional isoforms  
 46 of *OBSCN* is similar to the identification of exons within *TTN* that were thought to only occur in the

1 meta-transcript but were later shown to be present in *TTN* transcripts in adult skeletal muscle.<sup>46</sup> Our  
2 findings suggest that further studies are needed to provide a comprehensive picture of the complex  
3 *OBSCN* splicing pattern. As already demonstrated with the even larger *TTN* transcripts, this is crucial for  
4 a proper clinical interpretation of variants in such large genes.<sup>47</sup>

5 Obscurin was originally identified as a titin-binding protein and has been observed to localise to the M-  
6 band and also the Z-disk of striated muscle.<sup>25</sup> At the M-band, obscurin interactions with titin, myomesin  
7 and myosin binding protein C. Binding at the titin C-terminus Ig domain (M10) is responsible for the  
8 predominant M-band localisation in mature myofilaments. Heterozygous variants in the titin M10 domain  
9 cause dominant tibial muscular dystrophy (TMD)<sup>48</sup> and bi-allelic variants cause LGMD R10.<sup>49</sup> These  
10 variants disrupt binding with obscurin. TMD variants associated with different clinical severity, correlate  
11 with the degree of loss of obscurin interaction.<sup>25</sup>

12 Obscurin's precise role in skeletal muscle development, function and disease has remained *obscure*.<sup>50</sup> It  
13 had been shown in *C. elegans*, *D. Melanogaster* and *D. Rerio* that obscurin and its homologue unc-89  
14 might play an important role in sarcomerogenesis and the lateral alignment of sarcomeres.<sup>51-53</sup>

15 Four obscurin isoforms have been characterised, including two high-molecular weight proteins (obscurin  
16 A and B) that are abundant in skeletal muscle.<sup>28</sup> Obscurin A contains two COOH-terminal binding sites  
17 that can interact with ankyrin proteins<sup>54</sup>, including small ankyrin 1 (sAnk1.5) of the sarcoplasmic  
18 reticulum (SR). Obscurin is thus proposed to play a key role linking the contractile apparatus to the SR.  
19 *Obscn* null<sup>27</sup> and *sAnk1.5* null<sup>55</sup> mice both show reduced longitudinal SR volume. Muscle from patient  
20 FIN1 showed markedly dilated T-tubules by electron microscopy, this may indicate that the SR is  
21 impaired in obscurin-related myopathy.

22 Despite the great variability of causes, genetic or otherwise, rhabdomyolysis is thought to have the same  
23 downstream mechanism. Shortage of energy results in pump dysfunction (Na/K-ATPase, Ca<sup>2+</sup> ATPase  
24 pump), which leads to increased cellular permeability to Na<sup>+</sup> and an increased intracellular Ca<sup>2+</sup>  
25 concentration.<sup>56</sup> High intracellular Ca<sup>2+</sup> levels enhance the activation of Ca<sup>2+</sup>-dependent proteases and  
26 phospholipases, which contribute to the destruction of myofibrillar, cytoskeletal, and membrane proteins  
27 and leakage of myofibre contents, such as electrolytes, creatine kinase and myoglobin, into the  
28 circulation.<sup>57</sup> Aberrant Ca<sup>2+</sup> flux into cytosol and cell death are the hallmarks of rhabdomyolysis.<sup>58</sup> SR Ca<sup>2+</sup>  
29 contents are likely to be affected in rhabdomyolysis. In fact, SR Ca<sup>2+</sup> contents depletion, may reflect a  
30 lower level of SERCA activity and/or an increased Ca<sup>2+</sup> leak and this has two major consequences. Firstly,  
31 skeletal muscle contraction mainly relies on the amount of Ca<sup>2+</sup> released from SR and, as observed in  
32 heart failure,<sup>59, 60</sup> a decrease in SR Ca<sup>2+</sup> contents is associated with smaller amplitude and slower kinetics in  
33 cytosolic Ca<sup>2+</sup> transients as well as slower SR Ca<sup>2+</sup> reuptake. Therefore, repetitive muscle contractions in  
34 patients with rhabdomyolysis may lead to rapid exhaustion due to reduced SR Ca<sup>2+</sup> contents as well as  
35 slower kinetics of Ca<sup>2+</sup> release and repumping. Secondly, decreased SR contents is associated with stress  
36 disturbing folding of proteins related to the adaptative mechanism called the unfolded protein response  
37 (UPR) aimed to clear unfolded proteins and restore SR homeostasis.<sup>61</sup> SR stress often promotes apoptosis  
38 and lead to cell death as observed in rhabdomyolysis patients. Study of *OBSCN* null cell-lines (derived  
39 from mouse models, knock-down of *OBSCN* or patients), are now needed to strengthen these  
40 observations, including restoration of normal SERCA activity and measurements of Ca<sup>2+</sup> signals in  
41 skeletal muscle during repetitive electrical stimulation.

42 Studies by Lange and colleagues of *Obscn* null mice failed to identify any defects in sarcomere structure  
43 and alignment.<sup>27, 28</sup> In contrast, Sorrentino's team also generated and studied *Obscn* null mice, and found  
44 defects in sarcolemmal integrity and muscle damage in response to exercise.<sup>29, 30</sup> No defects suggestive of  
45 rhabdomyolysis or impaired Ca<sup>2+</sup> handling were noted in these mice studies; however it is tempting to

1 postulate that muscle damage triggered by obscurin deficiency, may lead to rhabdomyolysis  
2 susceptibility. Indeed, four of our six patients experienced rhabdomyolysis following exercise, in some  
3 instances mild exercise was sufficient to trigger an episode, i.e. climbing a flight of stairs. In the future, it  
4 may be of interest to explore the susceptibility of *Obscn* null mice to exercise and/or heat-induced muscle  
5 damage.

6 Our patients are relatively young, it will be interesting to follow up this cohort and identify additional  
7 cases with obscurin deficiency to determine the natural history and progression of the disease into later  
8 life.

9 Heterozygous variants (missense, splice-site and frameshift) in *OBSCN* have been associated with  
10 cardiomyopathy (dilated cardiomyopathy, hypertrophic cardiomyopathy, left ventricular non-compaction)  
11 in patients.<sup>40, 62, 63</sup> However, as highlighted in Grogan and Kontrogianni-Konstantopoulos the functional  
12 consequences of the identified *OBSCN* variants remain elusive.<sup>64</sup> More recently, a study by Fukuzuwa *et*  
13 *al.* has provided compelling evidence against the pathogenicity of one of the more widely-studied *OBSCN*  
14 variants (p.Arg4344Gln).<sup>65</sup> This study found no functional deficits associated with this variant (including  
15 studies to assess protein-protein interactions and thermostability).<sup>65</sup> In addition, 15% of African  
16 Americans were noted to carry this variant.<sup>65</sup> *Obscn*<sup>-/-</sup> mice do not exhibit any signs of cardiomyopathy.<sup>27</sup>  
17 In GTEx, *OBSCN* is highly enriched in skeletal muscle (median TPM 213) compared to all other tissues  
18 and is expressed at much lower levels in the heart (left ventricle: median TPM 35, atria appendage:  
19 median TPM 24; <https://www.gtexportal.org/home/gene/OBSCN>). In a systematic review of  
20 cardiomyopathy genetics, Ingles *et al.* classified the association between *OBSCN* variants and dilated  
21 cardiomyopathy as “limited” based on the available literature and scientific evidence.<sup>66</sup> None of the  
22 patients in this cohort nor any of their carrier first-degree relatives report any cardiac involvement,  
23 suggesting that loss-of-function *OBSCN* variants are unlikely to represent a substantial cause of  
24 cardiomyopathy.

25 In summary, we have identified bi-allelic loss-of-function variants in *OBSCN* predisposing individuals to  
26 recurrent rhabdomyolysis, typically presenting in teenage years. *OBSCN* should be considered in the  
27 genetic diagnosis of rhabdomyolysis.

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## 10 **Competing interests**

11 The authors report no competing interests.

## 12 **Supplementary material**

13 Supplementary material is available at *Brain* online.

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## 1 **Figure legends**

2 **Figure 1: Pedigrees for the six families segregating bi-allelic loss-of-function variants in *OBSCN***  
 3 **with predisposition to recurrent rhabdomyolysis.**

4 **Figure 2: Muscle pathology, by light and electron microscopy (EM), associated with bi-allelic null**  
 5 ***OBSCN* variants.** Features ranged from within normal limits to mild myopathic changes with increased  
 6 variation in myofibre size and internal nuclei (**A**: AUS1 and **J**: TUR1 [H&E], **B**: AUS2 and **C**: UK1 [Gomori  
 7 trichrome]). Glycogen accumulations are evident in skeletal muscle from FIN1 (**D**; periodic acid-Schiff  
 8 staining) and USA1 (**E**, EM). Prominent central cores are seen in type I myofibres of AUS2 (**F**,  
 9 NADH(blue)-fast myosin(brown) combined enzyme-immunohistochemistry and **G**, EM). **K**: NADH  
 10 staining on TUR1 shows mild disruption of internal architecture, type I myofibres appear almost  
 11 lobulated and occasional small core-like areas. Slow (**L**) and fast (**M**) myosin staining in TUR1 shows mild  
 12 predominance of type II myofibres, type I myofibres relatively small and few intermediate myofibres.  
 13 Dilated SR and t-tubules are also evident in the muscle biopsy of FIN1 (**H**; EM). Focal Z-band streaming is  
 14 seen in skeletal muscle from AUS1 (**I**; EM). Scale bars: 20  $\mu\text{m}$  (C, D), 50  $\mu\text{m}$  (A, B, K, J) 100  $\mu\text{m}$  (F, L, M), 2  
 15  $\mu\text{m}$  (G, H, I).

16 **Figure 3: Obscurin transcript and protein abundance in healthy control and patient**  
 17 **skeletal muscle.** (**A**) A schematic showing exon usage of exon 21 and 105, generated from  
 18 skeletal muscle RNA-seq data. (**B**) Transcript abundance of *OBSCN*, *RYR1*, *ACTA1* and *MYOG*  
 19 in human skeletal muscle obtained from the patient (filled square, grey bars) and five unrelated  
 20 controls (open circles, white bars) was assessed by qPCR. *OBSCN* transcript abundance is  
 21 specifically reduced by more than 10-fold in AUS1 patient skeletal muscle relative to controls.  
 22 Expression of each transcript was normalised to the geometric mean of two endogenous control  
 23 genes (*EEF2* and *TBP*) using the delta Ct method. Graphed data represent the *mean*  $\pm$  *SEM*. (**C**)  
 24 Western blot showing reduction/absence of OBSCN in patient muscle (FIN1, AUS1) compared  
 25 to healthy control (CTRL) samples. Coomassie staining of the TTN and NEB protein bands are  
 26 shown to demonstrate loading of total muscle protein.

27 **Figure 4: Studies from patient (UK1) myoblasts show aberrant  $\text{Ca}^{2+}$  flux and increased cell death.**  
 28 (**A**) SR morphology is not altered in patient myoblasts when compared to control myoblasts as shown by  
 29 immunostaining with anti-calnexin antibody and confocal analysis. (**B**) Total SR content and the  
 30 morphological parameters measured (length, sphericity) are similar in healthy control (CTRL) and patient  
 31 myoblasts. (**C**) Representative SR  $\text{Ca}^{2+}$  content measurements in myoblasts from a healthy control  
 32 (CTRL, left panel) and patient (right panel) in control (GM, black traces) and EBSS medium for 2 hours

1 (red traces). **(D)** SR Ca<sup>2+</sup> content was assessed from the area under the curves (AUC) after thapsigargin  
2 addition. Horizontal bar, 0 seconds; vertical bars F/Fmax 0.1 (arbitrary units). Histograms summarising  
3 area under the curve (AUC) in control (GM) and EBSS media. Data from 12 and six individual wells for  
4 CTRL and patient respectively obtained from two independent experiments corresponding to a decrease  
5 of 33±2 % (CTRL) and 69±6 % (Patient) of SR Ca<sup>2+</sup> contents in EBSS medium. **(E)** Apoptosis was  
6 assessed from purple events representing caspase 3/7 positive cells normalised to cell number. Patient  
7 myoblasts show higher levels of apoptosis (2.3-fold) as detected by caspase 3/7 expression when  
8 compared to CTRL myoblasts. **(F)** Quantification of caspase 3/7 expression in control and patient  
9 myoblasts. Results of one representative experiment out of two independent experiments.

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1 Table I Clinical details of six probands with OBSCN variants

ID	Age/sex (Age at 1st episode)	Onset of muscle symptoms and basal neurological examination	Peak CK (IU/l)/ basal CK between episodes	Rhabdo trigger	No. of episodes	Muscle pathology	Acyl carnitine profiles and or ischaemic/non-ischaemic forearm test results	LL Muscle MRI	Cardiac MRI	Exercise intolerance	Myalgia	Muscle weakness	Comparison syndrome	Renal failure
AUS1	20 y/M (18 y)	Exercise-induced myalgia and muscle cramps in childhood, mild distal weakness and exercise intolerance.	>500,000/~200-500	Heat, exercise	2	Mild random variation in myofibril size, increased central nuclei, occasional necrotic and regenerating myofibrils present.	Acyl carnitine - normal	Normal	Normal	Y	Y	Y (distal)	Y	Y
AUS2	39 y/M (27 y)	Presented for review after exercise intolerance and rhabdomyolysis. Normal on examination.	275,000/normal	Exercise	>2	Central core disease with fibre type variation	Acyl carnitine - normal	N/A	N/A	Y	Y	N	N	N
FIN1	38 y/M (15 y)	Occasional exercise-related myalgias in childhood	>90,000/normal-mildly elevated	Exercise	>2	Glycogen accumulation, dilated SR/T-tubules.	Acyl carnitine - normal	Normal	N/A	N	Y	N	N	N
TUR1	20 y/F (17 y)	Exercise-related myalgia and muscle cramps	>350,000/~400	Heat, exercise	2	Abnormal variation in myofibril size, increased internal nuclei. Some predominance of type 2 myofibr	N/A	N/A	N/A	Y	Y	N	N	Y

						es, lobulation of type I myofibr es, core- like regions.								
U KI	41 y/F (teen s)	Recurrent rhabdomyolysis	17,000/ normal	None	Up to 6/yr	Within normal limits	Acyl carnitine - normal	Unremarkable	Normal	N	N	N	N	N
US AI	19 y/M (12 y)	Exercise-related myalgia in childhood. Physically very active. Elite high school athlete (lacrosse)	603,000/ 500- 1000	None	3	N/A	Acyl carnitine - normal. Baylor metabolic testing normal. No ischaemic forearm test.	N/A	N/A	N	Y	N	Y	Y

1 CK = creatine kinase; F = female; LL = lower limb; M = male; N = no; SR/T = sarcoplasmic reticulum/Transverse; Y = yes.

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1 **Table 2 Details of the OBSCN variants identified in six recurrent rhabdomyolysis probands**

Patient	AUS1	AUS2		FINI		TURI	UKI		USA1	
Variant NM_00127122 3.2 obscurin isoform IC	exon 62: c.16230 C>A p.(Cys54 10*) - hmz	exon 21: c.6102 G>A p.(Trp2 034*)	exon 24: c.7078+ 1G>T p.(?)	exon36: c.9563_9576 del p.(Leu3188A rgfs*40)	exon105: c.23385_23 386del p.(Ser7796 *)	exon46: c.14818 C>T p.(Arg4 940*) - hmz	exon31: c.8253G >A p.(Trp27 51*)	exon42: c.11122 A>T p.(Lys37 08*)	exon2: c.386_387de linsAA p.(Phe129*)	exon90: c.21532+ 1G>A p.(?)
Null in isoform A/B <sup>a</sup>	Y/Y	N/N	Y/Y	Y/Y	N/Y	Y/Y	Y/Y	Y/Y	Y/Y	Y/Y
gnomAD allele freq	1.46 × 10 <sup>-5</sup>	1.21 × 10 <sup>-5</sup>	1.12 × 10 <sup>-4</sup>	0	3.79 × 10 <sup>-3</sup>	2.39 × 10 <sup>-5</sup>	0	8.04 × 10 <sup>-6</sup>	7.33 × 10 <sup>-4</sup>	1.58 × 10 <sup>-5</sup>
gnomAD hnz individuals	0	0	0	0	4	0	0	0	2	0
rs number	rs13223 44930	rs77577 2574	rs20084 9058	-	rs5362278 78	rs76681 4997	-	rs75853 6677	rs74956782 6 and rs76905058 8 (MNV)	rs750494 213

2

3 Hnz = homozygous; IC = inferred complete isoform; N = no; Y = yes.

4 <sup>a</sup>OBSCN transcript variant 1, NM\_052843.4, obscurin isoform A, OBSCN transcript variant 2, NM\_001098623.2, obscurin isoform B.

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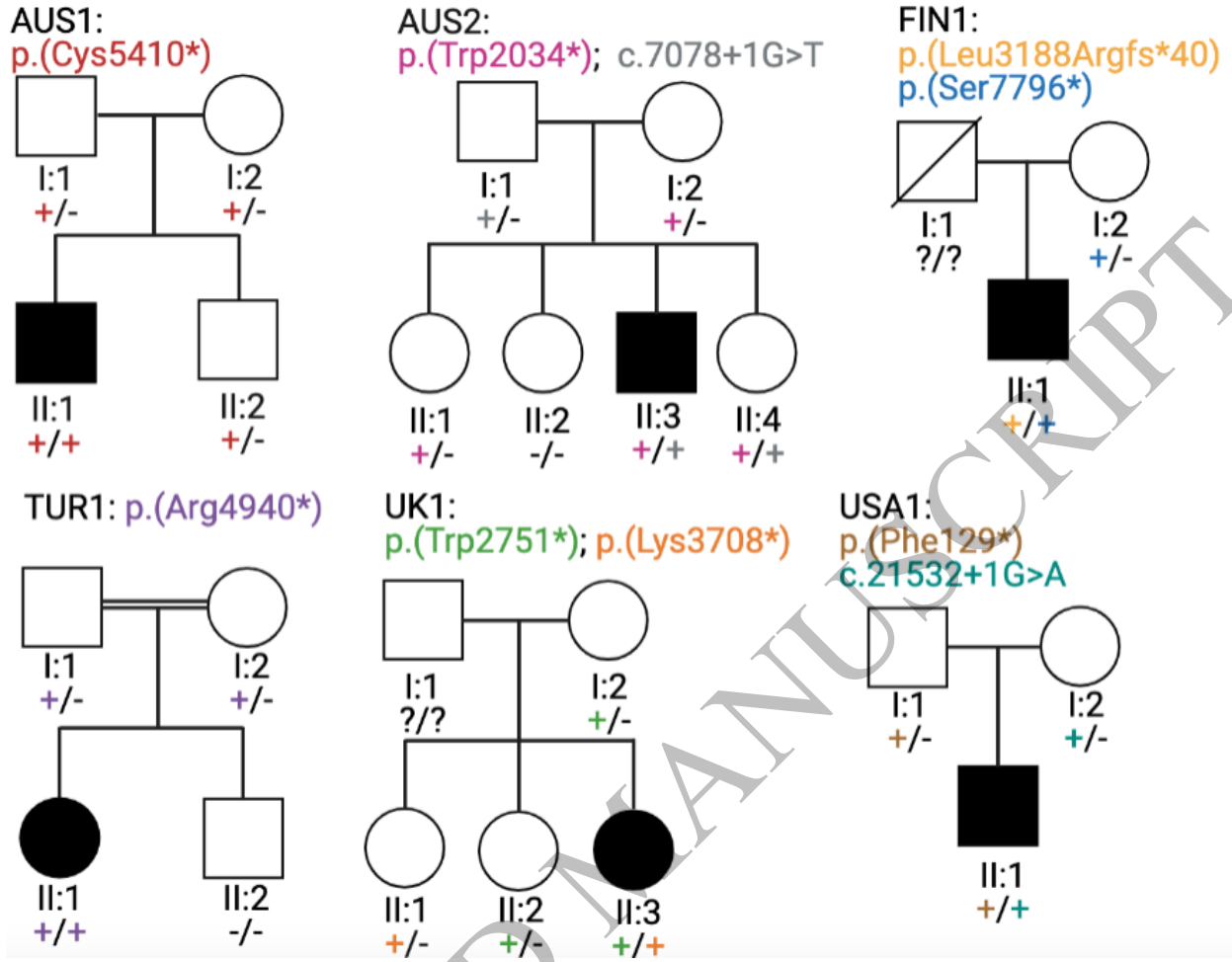


Figure 1  
165x128 mm (5.9 x DPI)

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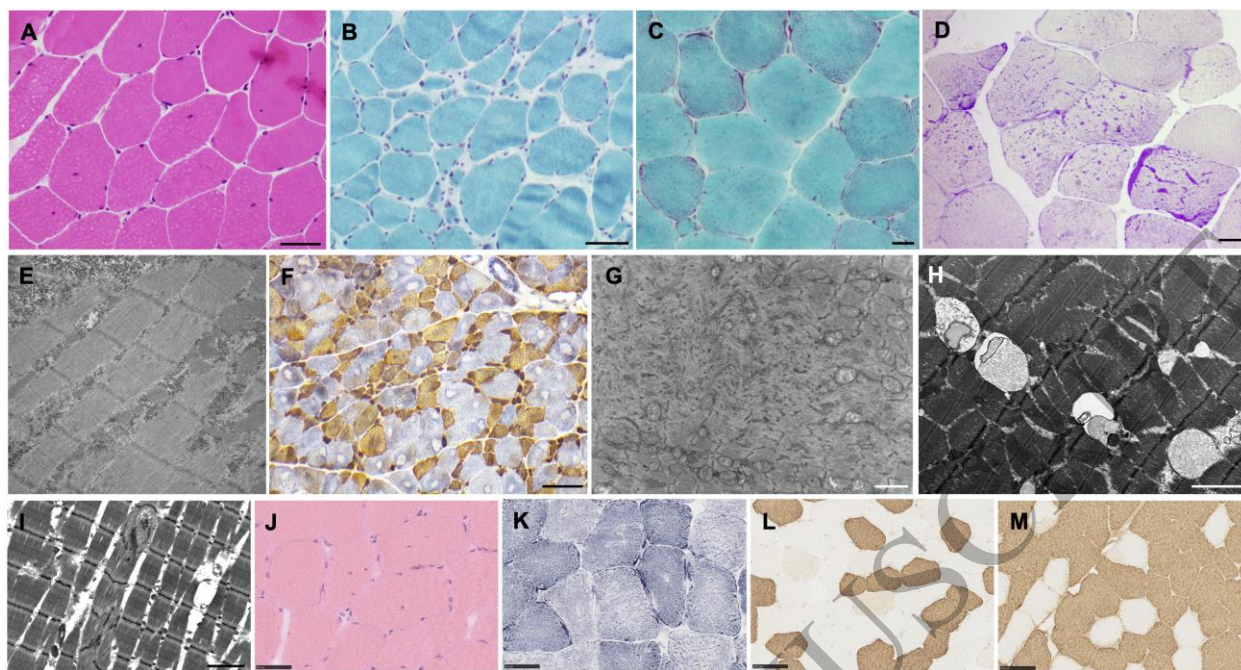


Figure 2  
165x88 mm (5.9 x DPI)

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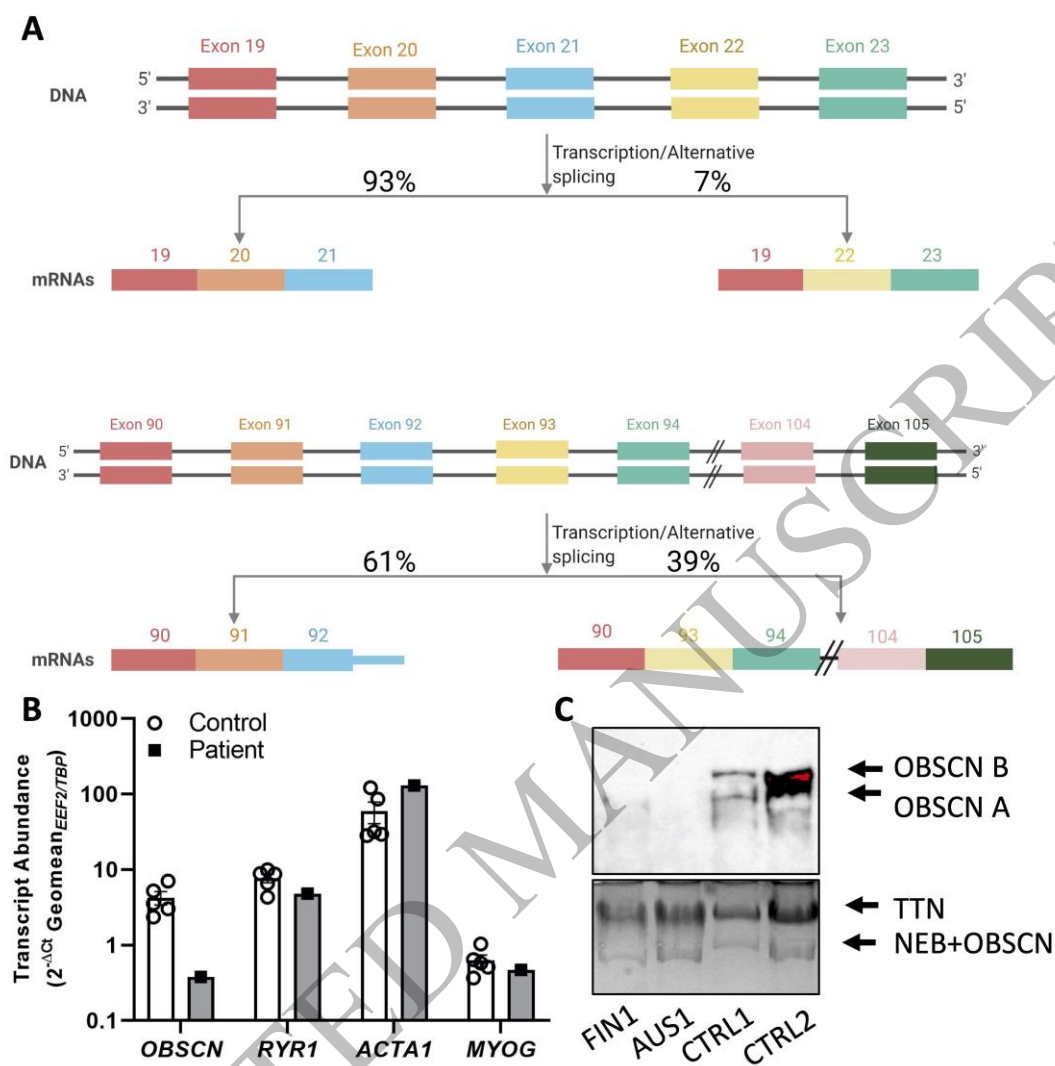


Figure 3  
144x150 mm (5.9 x DPI)

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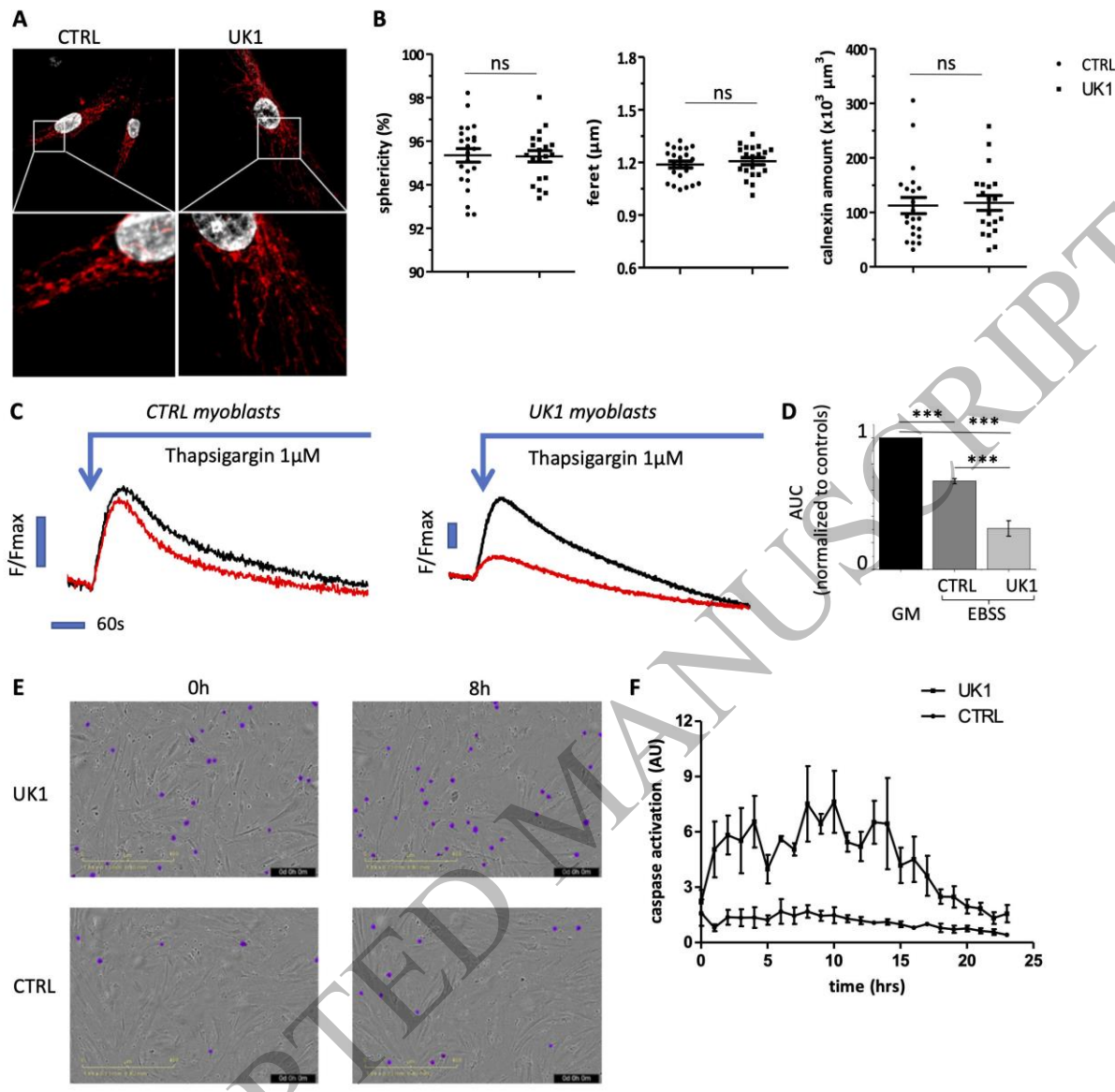


Figure 4  
165x155 mm (5.9 x DPI)

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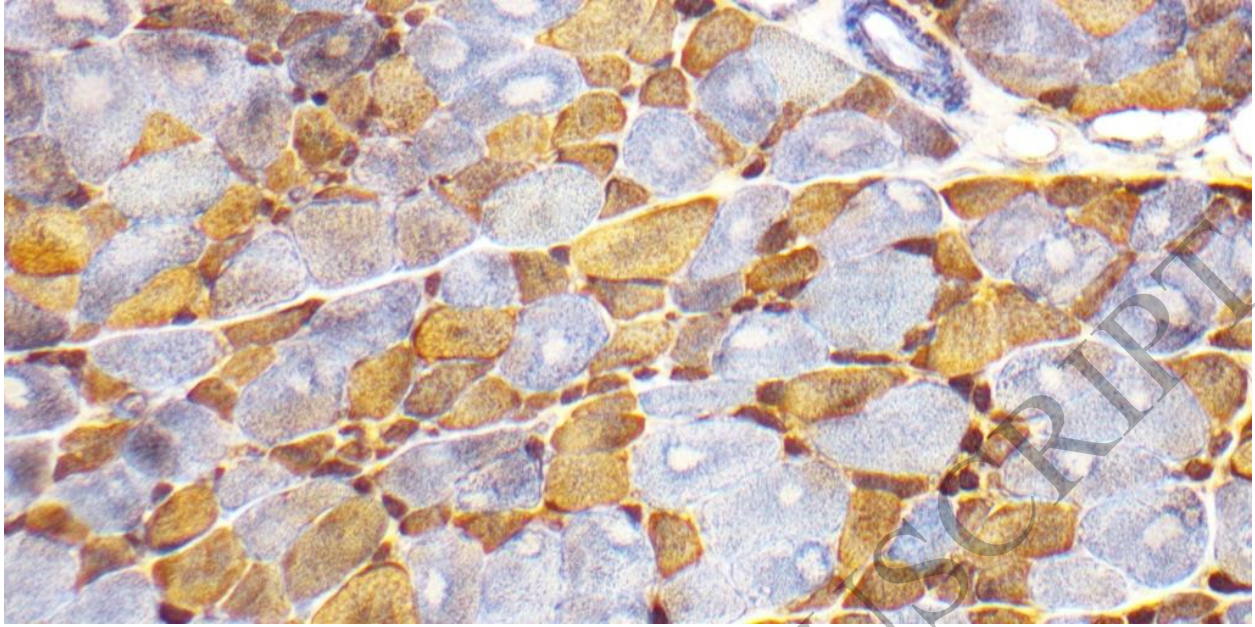


Figure 5  
165x83 mm (5.9 x DPI)

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1 Cabrera-Serrano *et al.* show that biallelic loss-of-function variants in the gene encoding obscurin  
2 (*OBSCN*) predispose individuals to recurrent and severe episodes of rhabdomyolysis, typically  
3 with onset in the teenage years.

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