Proper Voltage-Dependent Ion Channel Function in Dysferlin-Deficient Cardiomyocytes

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Key Words
Dilated cardiomyopathy • Dysferlin deficiency • Limb-girdle muscular dystrophy type 2B • L-type calcium channel • Mouse model • Ventricular cardiomyocytes • Voltage-dependent ion channels

Abstract:
Background/Aims: Dysferlin plays a decisive role in calcium-dependent membrane repair in myocytes. Mutations in the encoding DYSF gene cause a number of myopathies, e.g. limb-girdle muscular dystrophy type 2B (LGMD2B). Besides skeletal muscle degenerative processes, dysferlin deficiency is also associated with cardiac complications. Thus, both LGMD2B patients and dysferlin-deficient mice develop a dilated cardiomyopathy. We and others have recently reported that dystrophin-deficient ventricular cardiomyocytes from mouse models of Duchenne muscular dystrophy show significant abnormalities in voltage-dependent ion channels, which may contribute to the pathophysiology in dystrophic cardiomyopathy. The aim of the present study was to investigate if dysferlin, like dystrophin, is a regulator of cardiac ion channels. Methods and Results: By using the whole cell patch-clamp technique, we compared the properties of voltage-dependent calcium and sodium channels, as well as action potentials in ventricular cardiomyocytes isolated from the hearts of normal and dysferlin-deficient (dysf) mice. In contrast to dystrophin deficiency, the lack of dysferlin did not impair the ion channel properties and left action potential parameters unaltered. In connection with normal ECGs in dysf mice these results suggest that dysferlin deficiency does not perturb cardiac electrophysiology. Conclusion: Our study demonstrates that dysferlin does not regulate cardiac voltage-dependent ion channels, and implies that abnormalities in cardiac ion channels are not a universal characteristic of all muscular dystrophy types.
Introduction

Dysferlin is a member of the ferlin family of proteins. It is a membrane-spanning protein with multiple C2 domains that bind calcium, phospholipids, and proteins to set off signaling events, vesicle trafficking, and membrane fusion [1, 2]. In both skeletal and cardiac muscle cells dysferlin plays a decisive role in calcium-dependent membrane repair [3–5]. Consequently, injuries of the sarcolemma trigger the accumulation of dysferlin-enriched membrane patches and resealing of the membrane [3]. Mutations in the dysferlin gene (DYSF) result in the development of a number of progressive muscular dystrophies—the so-called “dysferlinopathies”, an example of which is limb-girdle muscular dystrophy type 2B (LGMD2B) [6]. Besides the relatively well characterized skeletal muscle degenerative processes, it has more recently become apparent that dysferlin deficiency is also associated with cardiac complications. Thus, both in LGMD2B patients [7, 8] and dysferlin-deficient mouse models [4, 7, 9] the development of a dilated cardiomyopathy was observed.

We and others have recently reported that dystrophin-deficient ventricular cardiomyocytes isolated from the hearts of mouse models of Duchenne muscular dystrophy (mdx, only dystrophin-deficient; mdx-utr, dystrophin- and utrophin-deficient) [10–13] show significant abnormalities in voltage-dependent ion channel expression and function. These abnormal “dystrophic” cardiac ion channel properties, i.e. enhanced L-type Ca\textsubscript{1.2} calcium currents [12, 13], slowed Ca\textsubscript{1.2} channel inactivation [12, 14, 15], and reduced Na\textsubscript{v}1.5 sodium currents [10, 11], may contribute to the pathophysiology in dystrophic cardiomyopathy. Direct regulation of Ca\textsubscript{1.2} channels by dystrophin accords with the finding that, in mouse cardiomyocytes, Ca\textsubscript{1.2} colocalizes with dystrophin [14].

In contrast to the established channel regulatory effects of dystrophin, regulation of Ca\textsubscript{1.2} and/or other cardiac voltage-dependent ion channels by dysferlin has not been studied as yet. Thus, it is unknown if dysferlin-deficiency impairs cardiac ion channel function, a potential pathophysiological mechanism in the dysferlinopathies. The following lines of evidence point to a potential regulatory effect of dysferlin on Ca\textsubscript{1.2} channels: First, in skeletal muscle, dysferlin colocalizes with the L-type calcium channel [16, 17], and muscle membrane repair requires an interplay between these two proteins [18]. Secondly, the dysferlin interaction partner AHNAK [19, 20] associates with regulatory β-subunits of Ca\textsubscript{1.2} channels [21, 22], and thirdly, the α\textsubscript{2}δ\textsubscript{1} calcium channel subunit, which modulates the channel’s function [23], is significantly downregulated in the dysferlin-deficient heart (NCBI GEO profile: http://www.ncbi.nlm.nih.gov/geo/; term=GDS1247[ACCN]+cacna2d1, ref. [24]). Finally, in the case of dysferlin absence from skeletal muscle cells, mechanical stress triggered abnormally increased calcium influx to the cytosol mediated by the L-type calcium channel, thereby disrupting calcium homeostasis and excitation-contraction coupling [25, 26]. To the best of our knowledge, there is no further evidence for potential regulatory effects of dysferlin on other cardiac ion channels than the L-type calcium channel as yet. In this study, we investigated if dysferlin-deficiency impairs cardiac electrophysiological properties by using a mouse model for LGMD2B.

Materials and Methods

Animal procedures adhered to the guiding principles of the Declaration of Helsinki and met the criteria of our Medical University’s Animal Welfare Committee. The Austrian Ministry for Science and Research granted the performed animal experiments (BMWF-66.009/0211-II/10b/2009).

Mouse models

Female wild type (wt) C57BL/10 mice, and dysferlin-deficient C57BL/10.SJL-Dysf mice [27, 28] in an age range between 15 and 20 months were used for the investigation. This advanced animal age was chosen because significant pathological changes are only present in the hearts of aged dysferlin-deficient mice [4]. Throughout the text, the dysferlin-deficient mouse model was termed “dysf”. The mice were genotyped by standard PCR-assays.
Isolation of adult ventricular cardiomyocytes

Mice were killed by cervical dislocation, and cardiomyocytes were isolated from the ventricles of their hearts by using a Langendorff setup as previously described [10]. Briefly, hearts were excised by thoracotomy and a cannula was inserted into the aorta to allow for retrograde perfusion with calcium-free solution (in mM: 134 NaCl, 11 glucose, 4 KCl, 1.2 MgSO₄, 1.2 Na₂HPO₄, 10 2, 3-butanedione monoxime, 10 Hepes, LiberaseTH (0.17 mg/ml) (Roche), pH adjusted to 7.35 with NaOH). Hearts were perfused for 18 min at 37°C. The ventricles were cut in pieces and incubated at 37°C on a shaker to stepwise increase the calcium concentration over one hour (final concentration, 200 µM). Digested tissue pieces were triturated to dissolve away cardiomyocytes. The cell suspension was then centrifuged (3 min, 500 rpm), the Langendorff solution was removed, and cells were resuspended in Minimum Essential Medium alpha (Gibco) with added: ITS media supplement (final compound concentrations: 10 µg/ml insulin, 5.5 µg/ml transferrin, 5 ng/ml selenite), 4 mM L-glutamine, 50 u/ml penicillin, 50 µg/ml streptomycin, and 25 µM blebbistatin. Cells were plated on dishes coated with Matrigel (Becton Dickinson). The chemicals originated from Sigma if not specified otherwise.

Cellular electrophysiological studies

Recordings of calcium, barium, and sodium currents, and of action potentials were made in the whole-cell configuration of the patch-clamp technique in the voltage- and current-clamp mode, respectively. A detailed description of the electrophysiological methods applied can be found in our previous studies [10,12]. Briefly, measurements were performed at room temperature (22 ± 1.5°C) up to 10 hours after isolation of ventricular cardiomyocytes. Glass pipettes pulled from aluminosilicate glass (AF150-100-10; Science Products) on a P-97 horizontal puller (Sutter Instruments) had series resistances between 0.8 – 1.5 MΩ when filled with the respective internal solutions and immersed into the bath. The compositions of the recording solutions (concentrations in mM) are given in Tables 1 and 2.

Voltage and current signals were recorded with an Axoclamp 200B patch-clamp amplifier (Axon Instruments), low-pass filtered at 1–10 kHz, and digitized at 10–100 kHz with a 12-bit A-D/D-A interface.

Table 1. External solutions

| Barium | Calcium | Sodium | Action potential |
|--------|---------|--------|-----------------|
| CaCl₂  | -       | 10     | 1               | 2               |
| BaCl₂  | 10      | -      | -               | -               |
| MgCl₂  | -       | -      | 1               | 2               |
| NaCl   | -       | -      | 15              | 140             |
| NMDG   | -       | -      | 125             | -               |
| KCl    | -       | -      | 2.5             | 4               |
| TEA-Cl | 145     | 145    | -               | -               |
| HEPES  | 10      | 10     | 10              | 5               |
| Glucose| -       | -      | -               | 5               |
| pH     | 7.4 (TEA-OH)| 7.4 (TEA-OH)| 7.4 (H-CI)| 7.4 (Na-OH) |

Table 2. Internal solutions

| Barium/Ca | Sodium | Action potential |
|-----------|--------|-----------------|
| Cs-aspartate | 145     | -               |
| CsF       | -      | 105             |
| KCl       | -      | -               |
| NaCl      | -      | 10              |
| MgCl₂     | 2      | -               |
| HEPES     | 10     | 10              |
| Cs-EGTA   | 0.1    | -               |
| Mg-ATP    | 2      | -               |
| Na-GTP    | -      | -               |
| EGTA      | -      | 10              |
| pH        | 7.4 (Cs-OH)| 7.3 (Cs-OH)| 7.2 (K-OH) |
Current-voltage (IV) relationships were fit using $I = \sum_{i=1}^{n} G_{\text{max},i} \cdot (V-V_{\text{rev},i}) \cdot (1-1/(1+\exp((V-V_{0.5,i})/K_{i})))$, with $n$ equaling 1 and 2 for barium and sodium, as well as calcium currents, respectively. $G_{\text{max},i}$, maximum conductance(s); $V$, membrane potential; $V_{\text{rev},i}$, reversal potential; $V_{0.5,i}$, voltage(s) of half-maximum activation; $K_{i}$, slope factor(s). Barium current inactivation kinetics (representing voltage-dependent calcium channel inactivation) was analyzed by fitting the current decay after channel activation with a single-exponential function to derive time constants ($\tau$-values). Calcium current inactivation kinetics (representing both calcium- and voltage-dependent calcium channel inactivation) was analyzed by measuring the time span between the peak current, and the time point at which the current had decayed to 50%. This parameter is termed “decay half-time”.

Action potentials (APs) were elicited both at 1 and 5 Hz by rectangular 4-ms current pulses at 125% threshold level. The maximum upstroke velocity (max. $dV/dt$) was determined at the maximum of the voltage signal’s time derivative. AP duration was analyzed from the maximum depolarization value reached to the time point at which 25% (APD25) and 75% (APD75) of repolarization had been achieved. AP area was determined by integration from the start of the current stimulus to the end of repolarization and is given in mV*ms.

**Statistical analyses**

Data are expressed as means ± SEM. Statistical comparisons between wt and dysf mice were made using an unpaired two-tailed Student’s t-test. A $p$ value $< 0.05$ was considered significantly different *; $p < 0.01$ highly significant difference **.
of the current decay after L-type calcium channel activation in wt and dysf cardiomyocytes revealed no significant differences. This was true for barium (Fig. 1C) and calcium (Fig. 1F) currents, suggesting that both voltage-dependent and calcium-dependent channel inactivation were similar in wt and dysf cardiomyocytes. Together these data suggested that dysf cardiomyocytes do not show calcium channel abnormalities.

Table 3. Calcium channel properties. $V_{0.5}$, voltage of half-maximum activation; $K$, slope factor; $V_{rev}$, reversal potential; $n$, number of experiments performed. T- and L-Type calcium current data points (Fig. 1E) were fit separately (for equation see Materials and Methods). No significant difference in any of the current parameters existed between wt and dysf cardiomyocytes ($p$-values > 0.05).

|        | $V_{0.5}$ (mV) | $K$ (mV) | $V_{rev}$ (mV) | $n$ |
|--------|----------------|----------|---------------|-----|
|        |                 |          |               |     |
| Barium |                |          |               |     |
| currents|                |          |               |     |
| wt     | L-type         | 0.8 ± 0.9| 5.7 ± 0.6     | 61 ± 1.5 | 15 |
| dysf   | L-type         | 3.8 ± 0.8| 5.5 ± 0.5     | 62 ± 1.3 | 13 |
| Calcium|                |          |               |     |
| currents|                |          |               |     |
| wt     | T-type         | -24 ± 2.4| 5.5 ± 0.9     | -    | 11 |
| L-type |                | 16 ± 1.8 | 8.4 ± 1.3     | 83 ± 2.6 | 11 |
| dysf   | T-type         | -24 ± 3.7| 5.5 ± 1.4     | -    | 13 |
| L-type |                | 16 ± 2.2 | 7.3 ± 1.5     | 86 ± 4.3 | 13 |

Fig. 1. Calcium channel properties in wild type (wt) and dysf cardiomyocytes. A. Original traces of barium currents of a typical wt cardiomyocyte elicited by the pulse protocol given on top. B. Current density-voltage relationships of wt and dysf cardiomyocytes (left). On the right, a comparison of the current densities at +20 mV is shown ($p$=0.44). C. Comparison of the barium current decay kinetics between wt and dysf cardiomyocytes at various membrane voltages ($p$ always>0.35). $\tau$-values were derived from single-exponential fits of the current decay after channel activation. D. Original traces of calcium currents of a typical wt cardiomyocyte elicited by the pulse protocol displayed on top. E. Current density-voltage relationships (left) and respective current densities for T-type (at -20 mV) and L-type (at +30 mV) calcium channels (right) in wt ($n$=11) and dysf (13) cardiomyocytes. F. Calcium current decay kinetics (decay half-times) in wt and dysf cardiomyocytes at various voltages ($p>0.16$). Fitting the data points of the current-voltage relationships in B and E revealed the parameters given in Table 3.
Sodium channels in wt and dysf cardiomyocytes

Dystrophin-deficient cardiomyocytes derived from mdx and mdx-utr mice showed significantly reduced sodium current densities [10, 11, 29]. Here we tested if the sodium currents were also reduced in cardiomyocytes from dysf mice. On the contrary, we found roughly similar current density–voltage relationships in wt and dysf cardiomyocytes (Fig. 2B), whereby a minor \( V_{0.5} \) shift toward more positive voltages in dysf cells showed up. The current densities analyzed at -25 mV (near the current maxima) were similar in wt and dysf cardiomyocytes. There was no significant difference (p=0.32).

Action potentials in wt and dysf cardiomyocytes

The cardiomyocyte’s action potential (AP) reflects the concerted action of a plethora of different types of ion channels. To detect any potential differences in ion channel...
properties between wt and dysf cardiomyocytes, we therefore also studied and compared their APs. Significant AP abnormalities were previously reported for dystrophin-deficient cardiomyocytes [10, 11]. Fig. 3 shows that the APs recorded from wt and dysf myocytes at two different frequencies (1 and 5 Hz) were similar. Thus, there was no significant difference between wt and dysf in any of the AP parameters analyzed. Parameters mainly reflecting sodium channel activity, i.e. AP upstroke velocity and peak AP amplitude (Figs. 3B and E), were as well similar as AP duration (Figs. 3C and F), which is essentially determined by the activity of L-type calcium and various potassium channels. Since calcium channel properties were normal in dysf cardiomyocytes (see above), our AP recordings indirectly suggested that the same is true for the ensemble of repolarizing potassium channels.

Electrocardiograms in wt and dysf mice

Similar ion channel and AP properties at the cardiomyocyte level accord with similar electrocardiogram (ECG) parameters in wt and dysf mice. In Fig. 4B, the QRS, the PQ, and the QT intervals of the ECG are compared between wt and dysf animals. It can be observed that all these parameters were normal in dysf mice. A normal QRS interval in dysf mice, reflecting the depolarization of the ventricles of the heart, corresponds to the normal AP upstroke velocity and peak AP amplitude observed in dysf cardiomyocytes (Figs. 3B and E). The ECG parameters likely to be affected by calcium channels are the PQ and the QT interval. These parameters reflect AV nodal conduction, and ventricular de- and repolarization, respectively. Our finding of similar PQ and QT intervals in wt and dysf animals thus accords with normal calcium channel properties in dysf cardiomyocytes (see above), our AP recordings indirectly suggested that the same is true for the ensemble of repolarizing potassium channels.

Discussion

Dysferlin is strongly expressed in cardiomyocytes [30] and plays a decisive role in calcium-dependent membrane repair [4]. Consequently, it does not surprise that dysferlin-deficiency is linked to cardiomyopathy development in mice [4, 7, 9] and in LGMD2B patients [7, 8]. Whereas dilated cardiomyopathy in the context of dystrophin-deficiency was associated with substantial cardiac voltage-dependent ion channel abnormalities [10–13], here we report basically normal ion channel properties in dysferlin-deficient (dysf) cardiomyocytes. Together with normal ECGs in dysf mice these results suggest that dysferlin-deficiency does not impair cardiac electrophysiology. Hence two important conclusions
can be drawn: 1) dysferlin, unlike dystrophin, does not regulate cardiac ion channels, and 2) significant abnormalities in cardiac voltage-dependent ion channels do not represent a universal characteristic of all types of muscular dystrophy.

A reasonable explanation for the lack of ion channel abnormalities in dysferlin- as opposed to dystrophin-deficient cardiomyocytes observed in the present study is yet to be found. A conspicuous difference between dysferlin- [4, 9, 31] and dystrophin-deficient [32–36] dystrophic mice is that the latter develop a more severe dilated cardiomyopathy. It is therefore tempting to speculate that the incidence of considerable cardiac electrophysiological abnormalities in a dystrophy mouse model is associated with the severity of the developing dilated cardiomyopathy. An argument supporting this hypothesis can be derived from our earlier work (Koenig et al. 2011 [10]), as well as from Albesa et al. 2011 [11]. Thus, in both studies, more severe sodium channel impairments were found in mdx-utr compared to mdx cardiomyocytes. The mdx-utr mouse model is known to develop a more pronounced dilated cardiomyopathy than mdx mice [33, 35, 36].

A surprising finding of the present study was that dysferlin, unlike dystrophin, does not regulate cardiac L-type calcium channels. We had expected a regulatory effect because the dysferlin interaction partner AHNAK [19, 20] associates with regulatory β-subunits of Ca\(_{\text{1.2}}\) channels [21, 22], and the α\(_{\text{1.2}}\)δ\(_{\text{1}}\) calcium channel subunit, which modulates the channel's function [23], is significantly downregulated in the dysferlin-deficient heart [24]. In this regard it is worthwhile to consider recent work dealing with dysferlin regulation of calcium signaling and homeostasis in skeletal muscle cells [25, 26]. It was suggested that dysferlin is a t-tubule protein that stabilizes stress-induced calcium signaling. According to that, mechanical stress results in influx of calcium to the cytosol mediated by the L-type calcium channel (adult skeletal muscle isoform, Ca\(_{\text{1.1}}\)) [25, 26]. This calcium influx does not cause significant muscle injury in wt muscle cells. However, when dysferlin is absent, calcium influx is greatly enhanced, disrupting calcium homeostasis and excitation-contraction coupling. Finally, this activates a cascade of calcium-mediated events promoting further damage to the muscle cell including proteolysis and oxidative stress [26]. A significant contribution of L-type calcium channels as pathway for deleterious calcium entry in dysferlin-deficient skeletal muscle cells was proven by showing that the L-type channel blocker diltiazem mitigated damage mediated by mechanical stress [25]. To our knowledge, it is currently unknown if mechanical stress also triggers abnormal calcium entry via L-type calcium channels in dysferlin-deficient cardiomyocytes. If so, it is conceivable that calcium channel function under conditions without mechanical stress is normal in dysferlin-deficient cells. This is in accordance with the findings of the present study. Under mechanical stress, however, L-type calcium channel activity in dysferlin-deficient cardiomyocytes may be abnormally enhanced as in dysferlin-deficient skeletal muscle cells [25, 26]. This could represent a mechanism to explain why dysferlin-deficient mice develop more pronounced cardiac abnormalities when their hearts are challenged by mechanical stress [4, 7].

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**Disclosure Statement**

No conflict of interest.
References

1. Anderson LV, Davison K, Moss JA, Young C, Cullen MJ, Walsh J, Johnson MA, Bashir R, Britton S, Keers S, Argov Z, Mahjineh I, Fougerousse F, Beckmann JS, Bushby KM: Dysferlin is a plasma membrane protein and is expressed early in human development. Hum Mol Genet 1999;8:855-861.

2. Matsuda C, Aoki M, Hayashi YK, Ho MF, Arahata K, Brown RH, Jr: Dysferlin is a surface membrane-associated protein that is absent in Miyoshi myopathy. Neurology 1999;53:1119-1122.

3. Bansal D, Miyake K, Vogel SS, Groh S, Chen CC, Williamson R, McNeil PL, Campbell KP: Defective membrane repair in dysferlin-deficient muscular dystrophy. Nature 2003;423:168-172.

4. Han R, Bansal D, Miyake K, Muniz VP, Weiss RM, McNeil PL, Campbell KP: Dysferlin-mediated membrane repair protects the heart from stress-induced left ventricular injury. J Clin Invest 2007;117:1805-1813.

5. Lek A, Evesson FJ, Sutton RB, North KN, Cooper ST: Ferlins: regulators of vesicle fusion for auditory neurotransmission, receptor trafficking and membrane repair. Traffic 2012;13:185-194.

6. Bansal R, Britton S, Strachan T, Keers S, Vafiadaki E, Lako M, Richard I, Marchand S, Bourg N, Argov Z, Sadeh M, Mahjineh I, Marconi G, Passos-Bueno MR, Moreira Ede S, Zatz M, Beckmann JS, Bushby K: A gene related to Caenorhabditis elegans spermatogenesis factor fer-1 is mutated in limb-girdle muscular dystrophy type 2B. Nat Genet 1998;20:37-42.

7. Wenzel K, Geier C, Qadri F, Hubner N, Schulz H, Erdmann B, Gross V, Bauer D, Dechend R, Dietz R, Osterziel KJ, Spuler S, Ozcelik C: Dysfunction of dysferlin-deficient hearts. J Mol Med 2007;85:1203-1214.

8. Koenig X, Deysek S, Kimbacher S, Mike AK, Cervenka R, Lukacs P, Nagl K, Fang XD, Todt H, Bittner RE, Hilber K: Voltage-gated ion channel dysfunction precedes cardiomyopathy development in the dystrophic heart. PLoS ONE 2011;6:e20300.

9. Albesa M, Ogrodnik J, Rougier JS, Abriel H: Regulation of the cardiac sodium channel Nav1.5 by utrophin in dystrophin-deficient mice. Cardiovasc Res 2011;89:320-328.

10. Koenig X, Rubi L, Obermaier GJ, Cervenka R, Dan GB, Kummer S, Bittner RE, Hilber K: Enhanced currents through L-type calcium channels in cardiomyocytes disturb the electrophysiology of the dystrophic heart. Am J Physiol Heart Circ Physiol 2013;304:H564-H573.

11. Li Y, Zhang S, Zhang X, Li J, Ai X, Zheng L, Yu D, Ge S, Peng Y, Chen X: Blunted cardiac beta-adrenergic response as an early indication of cardiac dysfunction in Duchenne muscular dystrophy. Cardiovasc Res 2014;103:60-71.

12. Sadeghi A, Doyle AD, Johnson BD: Regulation of the cardiac L-type Ca2+ channel by the actin-binding proteins alpha-actinin and dystrophin. Am J Physiol Cell Physiol 2002;282:C1502-C1511.

13. Viola HM, Davies SM, Filipowska A, Hoof LC: L-type Ca2+ channel contributes to alterations in mitochondrial calcium handling in the mdx ventricular myocyte. Am J Physiol Heart Circ Physiol 2013;304:H767-H775.

14. Kinge L, Hurst J, Sewry C, Charlton R, Anderson L, Laval S, Chiu YH, Hornsey M, Straub V, Barresi R, Lochmüller H, Bushby K: Dysferlin associates with the developing T-tubule system in rodent and human skeletal muscle. Muscle Nerve 2010;41:166-173.

15. Lek A, Evesson FJ, Lecomter FA, Redpath GM, Lueders AK, Turnbull L, Whitchurch CB, North KN, Cooper ST: Calpains, cleaved mini-dysferlinC72, and L-type channels underpin calcium-dependent muscle membrane repair. J Neurosci 2013;33:5085-5094.

16. Huang Y, Laval SH, van RA, Baudier J, Benaud C, Anderson LV, Straub V, Deelder A, Frants RR, den Dunnen JT, Bushby K, van der Maarel SM: AHNAK, a novel component of the dysferlin protein complex, redistributes to the cytoplasm with dysferlin during skeletal muscle regeneration. FASEB J 2007;21:732-742.

17. Cacciottolo M, Belcastro V, Laval S, Bushby K, di BD, Nigro V: Reverse engineering gene network identifies new dysferlin-interacting proteins. J Biol Chem 2011;286:5404-5413.
21 Hohaus A, Person V, Behlke J, Schaper J, Morano I, Haase H: The carboxyl-terminal region of ahnak provides a link between cardiac L-type Ca\(^{2+}\) channels and the actin-based cytoskeleton. FASEB J 2002;16:1205-1216.
22 Davis TA, Loos B, Engelbrecht AM: AHNAK: The giant jack of all trades. Cell Signal 2014;26:2683-2693.
23 Obermair OJ, Tuluc P, Flucher BE: Auxiliary Ca\(^{2+}\) channel subunits: lessons learned from muscle. Curr Opin Pharmacol 2008;8:311-318.
24 Wenzel K, Zabojszcza J, Carl M, Taubert S, Lass A, Harris CL, Ho M, Schulz H, Hummel O, Hubner N, Osterziel KJ, Spuler S: Increased susceptibility to complement attack due to down-regulation of decay-accelerating factor /CD55 in dysferlin-deficient muscular dystrophy. J Immunol 2005;175:6219-6225.
25 Kerr JP, Ziman AP, Mueller AL, Muriel JM, Kleinhans-Welte E, Gumerson JD, Vogel SS, Ward CW, Roche JA, Bloch RJ: Dysferlin stabilizes stress-induced Ca\(^{2+}\) signaling in the transverse tubule membrane. Proc Natl Acad Sci U S A 2013;110:20831-20836.
26 Kerr JP, Ward CW, Bloch RJ: Dysferlin at transverse tubules regulates Ca\(^{2+}\) homeostasis in skeletal muscle. Front Physiol 2014;5:89.
27 Bittner RE, Anderson LV, Burikhardt E, Bashir R, Vafiadaki E, Raffelsberger T, Maerk I, Höger H, Jung M, Karbasiyan M, Storch M, Lassmann H, Moss JA, Davison K, Harrison R, Bushby KM, Reis A: Dysferlin deletion in SJL mice (SJL-Dysf) defines a natural model for limb girdle muscular dystrophy 2B. Nat Genet 1999;23:141-142.
28 von der HM, Laval SH, Cree LM, Haldane E, Pocock M, Wappler I, Peters H, Reitsamer HA, Höger H, Wiedner M, Oberndorfer F, Anderson LV, Straub V, Bittner RE, Bushby KM: The differential gene expression profiles of proximal and distal muscle groups are altered in pre-pathological dysferlin-deficient mice. Neuromuscul Disord 2005;15:863-877.
29 Gavillet B, Rougier JS, Domenighetti AA, Behar R, Boixel C, Ruchat P, Lehr HA, Pedrazzini T, Abriel H: Cardiac sodium channel Nav1.5 is regulated by a multiprotein complex composed of syntrophins and dystrophin. Circ Res 2006;99:407-414.
30 Luft FC: Dysferlin, dystrophy, and dilatative cardiomyopathy. J Mol Med (Berl) 2007;85:1157-1159.
31 Hornsey MA, Laval SH, Barresi R, Lochmuller H, Bushby K: Muscular dystrophy in dysferlin-deficient mouse models. Neuromuscul Disord 2013;23:377-387.
32 Quinlan JG, Hahn HS, Wong BL, Lorenz JN, Wenisch AS, Levin LS: Evolution of the mdx mouse cardiomyopathy: physiological and morphological findings. Neuromuscul Disord 2004; 14:491-496.
33 Janssen PM, Hiranandani N, Mays TA, Rafael-Portney JA: Utrophin deficiency worsens cardiac contractile dysfunction present in dystrophin-deficient mdx mice. Am J Physiol Heart Circ Physiol 2005;289:H2373-H2378.
34 van PM, van der Pijl EM, Hulsker M, Verhaart IE, Nadarajah VD, van der Weerd L, Artsma-Rus A: Low dystrophin levels in heart can delay heart failure in mdx mice. J Mol Cell Cardiol 2014;69:17-23.
35 Grady RM, Teng H, Nichol MC, Cunningham JC, Wilkinson RS, Sanes JR: Skeletal and cardiac myopathies in mice lacking utrophin and dystrophin: a model for Duchenne muscular dystrophy. Cell 1997;90:729-738.
36 Chun JL, O’Brien R, Berry SE: Cardiac dysfunction and pathology in the dystrophin and utrophin-deficient mouse during development of dilated cardiomyopathy. Neuromuscul Disord 2012;22:368-379.