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Electroretinogram Abnormalities in *FKRP* Related Limb-Girdle Muscular Dystrophy (LGMDR9)

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Abstract

Background: Dystroglycanopathies are a heterogenous group of membrane-related muscular dystrophies. The dystroglycanopathy phenotype includes a spectrum of severity ranging from severe congenital muscular dystrophy to adult-onset limb girdle muscular dystrophy (LGMD). LGMDR9 is a dystroglycanopathy caused by mutations in the *FKRP* gene. Previous studies have characterized electroretinogram findings of dystroglycanopathy mouse models but have not been reported in humans.

Purpose: This study set out to characterize the electroretinogram in eight participants with LGMDR9.

Methods: Eight participants were recruited from an ongoing dystroglycanopathy natural history study at the University of Iowa (NCT00313677). Inclusion criteria for the current study were children and adults > 6 years old with confirmed LGMDR9. Age similar controls were identified from our electrophysiology service normative control database. Full field electroretinograms were recorded using ISCEV standards. Six of the eight participants underwent light adapted ON/OFF testing.

Results: The electronegative electroretinogram was not seen in any participants with LGMDR9. An unusual sawtooth pattern in the 30 Hz flicker with faster rise than descent was noted in all 8 participants. Our cases showed a decreased b-wave amplitude in light adapted ON responses (p=0.011), and decreased d-wave amplitude in light adapted OFF responses (p=0.015). Decreased b-wave amplitude in 3.0 light adapted testing (p=0.015) and decreased flicker amplitudes were

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Code Availability: Not applicable

Ethics Approval: This prospective study was approved by the Institutional Review Board of the University of Iowa (IRB ID #201805774) and conformed to the requirements of the United States Health Insurance Portability and Privacy Act.

Conflicts of interests: Not applicable.

also detected (p=0.0018). Additionally, compared to controls, participants with LGMDR9 had decreased a-wave amplitudes on 10.0 dark adapted testing (p=0.026).

Conclusions: Abnormal ON/OFF bipolar cell responses and sawtooth 30 Hz flicker waveforms on full field electroretinogram may be specific for LGMDR9. If confirmed in a larger population and if related to disease stage, these tests are potential biomarkers which could be useful as endpoints in clinical treatment trials.

Keywords

Electroretinogram; dystroglycanopathy; electronegative; amplitudes; dystrophy; LGMDR9; Limb-Girdle Muscular Dystrophy; *FKRP*

Background/ Introduction:

Dystroglycanopathies, disorders of alpha-dystroglycan (α -DG) glycosylation, are a heterogenous group of membrane-related muscle autosomal recessive dystrophies that include both congenital muscle dystrophies and limb-girdle muscle dystrophies [1]. At the more severe end, the dystroglycanopathy phenotype includes a spectrum of abnormal ocular and neurodevelopmental findings while patients with milder forms of muscular dystrophies have no documented ocular or retinal abnormalities [2]. Alpha-dystroglycan (α-DG) is an extracellular component of the dystrophin-glycoprotein complex that binds to β-DG, a transmembrane glycoprotein. α-dystroglycan (α-DG) is a receptor for matrix and synaptic proteins; abnormal glycosylation of α-DG causes muscular dystrophy with or without brain and eye development. (α-dystroglycanopathies) [3]. Glycosylated α-Dystroglycan binds laminin, perlecan, agrin, neurexin, and pikachurin in the extracellular space [4–8], and the intracellular C-terminal tail of β-dystroglycan binds dystrophin in the cytoskeleton [4]. The physical link connecting the extracellular matrix and the cytoskeleton is mediated by proper glycosylation, mainly O-mannosylation of dystroglycan [9]. In the retina, dystroglycan is concentrated in the Müller glial end feet at the inner limiting membrane and in the glial end feet abutting the vasculature [10]. Dystroglycan is also expressed at ribbon synapses of rod and cone photoreceptors in the outer plexiform layer of the retina, which are important for propagating the b-wave in an electroretinogram [11, 12].

An attenuated b-wave has been found in mouse models of some dystroglycanopathies. It has been reported that certain mutations in beta-dystroglycan as well as the absence of dystroglycan in glial cells can result in an attenuated b-wave [13]. A recently developed *POMT1* deficient mouse model had impaired photoreceptor ribbon synapses and an electronegative b-wave on electroretinography [12]. In addition, a severely diminished b-wave amplitude has been found in mice with mutations in the *LARGE* gene [14]. Biallelic mutations in the *POMT1* and *LARGE* genes are known to cause dystroglycanopathies [11,13].

Mutations in *FKRP,* which encodes for fukutin-related protein, are one of the most common causes of dystroglycanopathy genes [15, 16]. *FKRP* mutations are associated with muscular dystrophies of highly variable clinical severity. Most mutations result in limb-girdle muscular dystrophy (LGMDR9), but *FKRP* mutations can also cause severe

forms of congenital muscular dystrophy (MDC1C) with or without involvement of central nervous system, Walker–Warburg syndrome and muscle-eye-brain disease [16, 17]. Fukutin-related protein has been shown to function as a ribitol 5-phosphate transferase, which is necessary for the glycosylation of alpha-dystroglycan [3, 18]. The purpose of this study was to characterize the electroretinogram in people with LGMDR9.

Methods:

Participants:

Participants were recruited from an ongoing prospective natural history study at the University of Iowa (NCT00313677). Research participants > 6 years old with confirmed LGMDR9 seen between 7/13/2018 and 8/4/2022 were invited to participate in this study of retinal function, depending on technician availability. Eight participants underwent electroretinogram testing. Five of these participants underwent light and dark adapted full field electroretinograms; an additional 3 participants underwent light adapted testing only. Light adapted ON/OFF testing was performed in 6 of the 8 participants. Average age of all 8 participants was 43.5 years old (range 22–56 years). The internal controls used for the full field electroretinogram testing (NL1–5) had an average age of 30.6 years old, ranging from 22 to 52 years old. Six (6) age-similar internal controls for ON/OFF testing (average age 36.3, range 23– 63) from our electrophysiology service were also used (NL 6–11). All controls had normal visual acuity (20/15–20/30) and no known ocular pathology which could affect electroretinogram amplitudes.

Experimental Protocol:

Our participants underwent penlight ophthalmic examination, best corrected near visual acuity, intraocular pressure measurement, and full field electroretinogram. Pupils were dilated with one drop of Tropicamide 1%. The unit of time-integrated luminance is the candela-second per square metre (cd·s/m^2). All brief flashes used in clinical electrophysiology are specified in cd·s/m^2. Participants underwent full field electroretinograms using ISCEV standards for full-field clinical electroretinography [19]. The electroretinograms were elicited in response to flash stimuli of strengths 0.009, 3.0 and 10.0 cd (candelas) • s/m^2 presented scotopically and 3.0 cd • s/m^2 presented photopically on a Ganzfeld background luminance of 34 cd/m^2. Participants were tested binocularly with 15 responses averaged per eye. The electroretinograms were acquired in a time window of 100–200 milliseconds, which included a 10-millisecond prestimulus interval. Oscillatory potentials (OPs) were filtered between 100 and 300 Hz. In addition, photopic 30-Hz flicker electroretinograms (30.30 Hz) to 3.0 photopic cd • s/m^2 flash strengths were recorded binocularly. Testing included the light-adapted 3.0 electroretinogram, light-adapted 30 Hz flicker, dark-adapted (DA) 0.01 electroretinogram, dark-adapted (DA) 3.0 electroretinogram, dark-adapted (DA) 10.0 electroretinogram, and dark-adapted (DA) 3.0 oscillatory potentials. Light adapted testing was performed first, followed by dark adapted testing after 25 minutes of dark adaptation. Full field electroretinogram testing was performed using the ColorDome (Diagnosys LLC, Lowell, MA) Ganzfield stimulator and was recorded using Dawson, Trick, and Litzkow (DTL) corneal fiber electrodes (Diagnosys LLC, Lowell, MA).

Six participants also underwent light adapted ON/OFF testing using ISCEV standards. Prolonged on and off flashes were presented using white stimulus (150 cd/m^2) on a white background under photopic conditions (30 cd/m^2). ON responses were recorded with ramping down waveform shape whereas OFF-responses were recorded with ramping up waveform shape [20]. This prospective study was approved by the Institutional Review Board of the University of Iowa (IRB ID #201805774) and conformed to the requirements of the United States Health Insurance Portability and Privacy Act.

Results:

Eight participants with *FKRP* mutations, five male and three female, were recruited to undergo study procedures. Five participants (Cases 1–5) underwent both light and dark adapted full field electroretinograms. Three participants (Cases 6, 7 and 8) completed only light adapted electroretinogram testing. Light adapted ON/OFF testing was performed on six (6) participants (Cases 1, 2, 3, 6, 7 and 8). Six participants were homozygous for the common c.826C>A mutation in *FKRP* while two had compound heterozygous variants in *FKRP* (Table I). All eight participants had normal visual acuity (20/15–20/30), and none reported any visual deficits. Intraocular pressures were within normal limits for five participants (Case 1–5); in three participants (Cases 6, 7 and 8) intraocular pressures could not be ascertained. None of our participants in this study had evidence of cataracts or ptosis.

As seen in Figure 1 and Table 2, electronegative waveforms in the dark-adapted 3.0 or 10.0 electroretinograms were not seen on any of our participants. B/A wave ratios ranged from 1.89 to 3.44 for the dark adapted 3.0 electroretinogram with an average of 2.49 and a standard deviation of 0.52. Two participants had b/a wave ratios < 2.0, while all others were above 2.0. The average 3.0 dark adapted b-wave amplitude in our participants was 306.01 microvolts (μV), compared to our controls with an average of 376.43 μV (p=0.22). The average 3.0 dark adapted a-wave amplitude of our participants was −120.43 μV, compared to −180.57 μV for our controls (p=0.077).

The control subjects were found to have significantly higher a-wave amplitudes on dark adapted 10.0 flashes than our participants with *FKRP* mutations (p=0.026). Our LGMDR9 s participants had an average 10.0 dark adapted a-wave amplitude of −113.34 μV and the controls had an average of −166.26 μV. Controls also had higher average dark-adapted 10.0 b-wave amplitudes, 273.3 μV compared to 239.02 μV for LGMDR9 participants, but this was not statistically significant.

Light adapted electroretinogram testing revealed a higher b and a wave amplitude in the controls than the LGMDR9 participants. Controls' average b-wave and a-wave amplitudes were 154.88 and −31.20 μV whereas LGMDR9 participants were 123.49 and −26.73 μV (Table III). Although both b and a-wave amplitudes were higher in the controls, only the b-wave amplitudes were statistically significant to p<0.05 (p=0.0155).

The average amplitude of the 30 Hz flicker for LGMDR9 participants was 86.58 μV compared to 118.18 μV for controls (p=0.002). All 8 participants exhibited the sawtooth waveform on 30 Hz flicker compared to no controls. Cases 1, 2, 4, 5, 6, and 8 exhibited the

most noticeable saw tooth waveform on the 30 Hz flicker with delayed latency to the first trough and broad trough between peaks due to a rapid rising limb of the peak and prolonged descending limb; Cases 3 and 7 had rapid rise but less prominent delayed latency (Figure 2).

For the 6 participants with ON/OFF response data, the average b-wave amplitude during the rapid on phase of testing was 59.70 μV for LGMDR9 participants and 80.43 μV for controls with a p-value of 0.011. The average a-wave amplitude during the on phase of testing was −31.31 μV for our participants compared to controls of −35.19 μV (p=0.415). The average amplitude for the Rapid OFF testing or d-wave was also noted to be significantly higher in controls (44.41 μV) than in LGMDR9 participants (27.44 μV) p= 0.015. Different age-similar internal controls were used for ON-OFF testing (NL 6–11) than for full field electroretinograms (Table 4, Figure 3).

Discussion:

We observed several differences in electroretinograms between LGMDR9 participants and normal controls. The most striking finding was the absence of the electronegative electroretinogram on scotopic testing and a “sawtooth” pattern in the 30 Hz flicker with faster rise than descent in participants compared to controls. We also observed a decreased b-wave amplitude using ON response testing and decreased d-wave amplitude using OFF responses testing. Although we did not find an electronegative electroretinogram on scotopic testing we did observe significantly lower a-wave amplitudes on 10.0 dark adapted flashes. We also noted a decreased b-wave amplitude in 3.0 light adapted testing and decreased amplitudes on photopic 30 Hz flicker in the LGMDR9 participants tested.

The a-wave of the electroretinogram is generated primarily by photoreceptors. In the dark adapted 3.0 intensity condition this includes both rod and cone photoreceptors, but the increase in amplitude with raising the intensity to 10.0 is mediated largely by rods. According to the ISCEV guide to visual electrodiagnostic procedures, ‘The dark adapted 3.0 (standard flash) and dark adapted 10.0 (strong flash) electroretinograms have input from both rod and cone systems, but the dark adapted rod system contribution dominates in a normal retina. The first 8ms of the cornea-negative a-wave reflects rod hyperpolarization, and as the a-wave in the dark adapted 10.0 electroretinogram is of shorter peak time and larger than in the DA 3.0 electroretinogram, it provides a better measure of rod photoreceptor function’ [21].

In all participants and controls, the b/a ratio decreased with the brighter 10.0 flash. This data is in line with a recent study in which 211 normal adults had electroretinogram b/a wave ratios measured and Mean (SD) b/a ratios for normal right and left eyes, respectively, were 1.86 (0.33) and 1.81 (0.29) for the standard flash, and 1.62 (0.25) and 1.58 (0.23) for the stronger flash. The average b/a ratio was lower for the stronger flash in all cases ($p<0.0001$) [22].

The average amplitude on the 30 Hz flicker for our dystroglycanopathy participants was significantly lower than our controls (p=0.002). Six of the eight participants had a pronounced 30 Hz “sawtooth” waveform pattern with faster rise than descent. This

"sawtooth" waveform pattern has been described before in other cases of congenital disorders of glycosylation [23]. They hypothesized that the site of retinal dysfunction in their case was at the cone photoreceptor synapse with the cone on-bipolar cell [23]. We hypothesize that cone bipolar cell dysfunction may be the location of dysfunction in our LGMDR9 participants. To investigate the abnormal 30 Hz flicker in more detail, we conducted light adapted ON/OFF testing in six dystroglycanopathy participants (Case 1, 2, 3, 6, 7, 8). We found that our participants had significantly decreased b-wave amplitudes during ON-response testing when compared to age similar controls as well as significantly decreased d-waves to OFF-response testing. This test specifically evaluates the ON and OFF bipolar cone synapses, and decreased amplitude indicates decreased function, consistent with our hypothesis. This concept of cellular dysfunction occurring in the retinal pathway proximal to the cones is not a new one, nor is using the electroretinogram to judge the involvement of photoreceptors (a-wave) versus cells postsynaptic to the cones (b- and d- wave). Isolated decreased On- or OFF- responses may suggest postsynaptic cone dysfunction that does not have to be due to cone photoreceptor loss. [24].

Duchenne and Becker muscular dystrophies (DBMD) result from defects in dystrophin. Dystrophin is an intracellular protein that forms a transmembrane complex (the dystrophin glycoprotein complex) through interactions with beta and alpha dystroglycan. Patients with DBMD have a well characterized abnormal response in the electroretinogram [25–28]. This unique finding, the electronegative b-wave, is used as a biomarker for dystrophin dysfunction, and has been suggested as a biomarker of disease progression [29]. The electronegative electroretinogram, which has been reported in humans with Duchenne and Becker muscular dystrophy and in mouse models of dystroglycan-deficiency, was not found in this study's human population with LGMDR9. None of the participants in this study had a b-wave/a-wave ratio of less than one.

When we look at summation of data from our participants, we see six meaningful results: a- waves in the DA 10.0 electroretinogram are lower amplitude in LGMDR9 participants, a decreased b- wave amplitude on light adapted ON response testing, a decreased d-wave amplitude on OFF-response testing, a LA 30 Hz flicker with a "sawtooth" pattern with a faster rise than descent, decreased b-wave amplitude on light adapted 3.0 testing, and the absence of the electronegative electroretinogram. This data suggests some level of On- and Off cone bipolar cell dysfunction in people with *FKRP*-related dystroglycanopathy.

Our current understanding of alpha-dystroglycan glycosylation at photoreceptor ribbon synapses would support our findings of On- cone bipolar cell dysfunction in these patients [8, 30, 31]. One study was able to identify the heparan sulfate proeoglycan (HSPG) member Pikachurin, released by the photoreceptors, recruited a key post-synaptic signaling complex of downstream ON-bipolar neurons in coordination with presynaptic dystroglycan glycoprotein complex. They demonstrated that the transsynaptic assembly played an essential role in synaptic transmission of photoreceptor signals. (27) Another study was recently published which evaluated retinal proteomics of *POMT1* knockout mice. They found the upregulation of a set of proteins that are commonly expressed in degenerating neurons prior to cell death. This cluster includes glial fibrillary acidic protein (GFAP), basic fibroblast growth factor (FGF2), signal transducer, and activator of transcription 3

(STAT3), αB-crystallin (CRYAB), and S100 calcium binding protein B (S100B) [32]. This may signify some level of photoreceptor stress, which might help explain the decrease in amplitudes seen in our single flash testing.

This exploratory study had limitations, the most important of which is small sample size due in part to the fact that dystroglycanopathies are rare disorders and limited number of normal controls. We accounted for this by having all tests performed by the same person (WLP) under the same conditions following ISCEV guidelines.

In this work, we found electroretinogram evidence to suggest dysfunction of the cone bipolar cells in participants with LGMDR9. It will be important to confirm these findings in additional patients, to determine the relationship to other measures of disease severity, and to determine the stability of the observations over disease progression. If the abnormalities observed are related to level of dystroglycan glycosylation and change over time, it is possible electroretinograms could be a non-invasive biomarker for future therapies intended to increase alpha dystroglycan glycosylation.

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Availability of data and materials:

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References

1. Muntoni F, et al. , Muscular dystrophies due to glycosylation defects: diagnosis and therapeutic strategies. Curr Opin Neurol, 2011. 24(5): p. 437–42. [PubMed: 21825985]
2. Taniguchi-Ikeda M, et al. , Mechanistic aspects of the formation of alpha-dystroglycan and therapeutic research for the treatment of alpha-dystroglycanopathy: A review. Mol Aspects Med, 2016. 51: p. 115–24. [PubMed: 27421908]
3. Kanagawa M, et al. , Identification of a Post-translational Modification with Ribitol-Phosphate and Its Defect in Muscular Dystrophy. Cell Rep, 2016. 14(9): p. 2209–2223. [PubMed: 26923585]
4. Ervasti JM and Campbell KP, A role for the dystrophin-glycoprotein complex as a transmembrane linker between laminin and actin. The Journal of cell biology, 1993. 122(4): p. 809–823. [PubMed: 8349731]
5. Gee SH, et al. , Dystroglycan-alpha, a dystrophin-associated glycoprotein, is a functional agrin receptor. Cell, 1994. 77(5): p. 675–86. [PubMed: 8205617]
6. Peng HB, et al. , The relationship between perlecan and dystroglycan and its implication in the formation of the neuromuscular junction. Cell Adhes Commun, 1998. 5(6): p. 475–89. [PubMed: 9791728]
7. Sugita S, et al. , A stoichiometric complex of neurexins and dystroglycan in brain. J Cell Biol, 2001. 154(2): p. 435–45. [PubMed: 11470830]
8. Sato S, et al. , Pikachurin, a dystroglycan ligand, is essential for photoreceptor ribbon synapse formation. Nat Neurosci, 2008. 11(8): p. 923–31. [PubMed: 18641643]

9. Yoshida-Moriguchi T. and Campbell KP, Matriglycan: a novel polysaccharide that links dystroglycan to the basement membrane. Glycobiology, 2015. 25(7): p. 702–13. [PubMed: 25882296]
10. Montanaro F, et al. , Dystroglycan expression in the wild type and mdx mouse neural retina: synaptic colocalization with dystrophin, dystrophin-related protein but not lamininJ Neurosci Res, 1995. 42(4): p. 528–38. [PubMed: 8568939]
11. Jastrow H, et al. , Identification of a beta-dystroglycan immunoreactive subcompartment in photoreceptor terminals. Invest Ophthalmol Vis Sci, 2006. 47(1): p. 17–24. [PubMed: 16384939]
12. Rubio-Fernandez M, et al. , Impairment of photoreceptor ribbon synapses in a novel Pomt1 conditional knockout mouse model of dystroglycanopathy. Sci Rep, 2018. 8(1): p. 8543. [PubMed: 29867208]
13. Satz JS, et al. , Visual impairment in the absence of dystroglycan. J Neurosci, 2009. 29(42): p. 13136–46. [PubMed: 19846701]
14. Holzfeind PJ, et al. , Skeletal, cardiac and tongue muscle pathology, defective retinal transmission, and neuronal migration defects in the Largemyd mouse defines a natural model for glycosylation-deficient muscle – eye – brain disorders. Human Molecular Genetics, 2002. 11(21): p. 2673–2687. [PubMed: 12354792]
15. Cohn RD, Dystroglycan: important player in skeletal muscle and beyond. Neuromuscul Disord, 2005. 15(3): p. 207–17. [PubMed: 15725582]
16. Brockington M, et al. , Mutations in the fukutin-related protein gene (FKRP) identify limb girdle muscular dystrophy 2I as a milder allelic variant of congenital muscular dystrophy MDC1C. Hum Mol Genet, 2001. 10(25): p. 2851–9. [PubMed: 11741828]
17. Beltran-Valero de Bernabé D, et al. , Mutations in the FKRP gene can cause muscle-eye-brain disease and Walker-Warburg syndrome. J Med Genet, 2004. 41(5): p. e61. [PubMed: 15121789]
18. Esapa CT, et al. , Functional requirements for fukutin-related protein in the Golgi apparatus. Hum Mol Genet, 2002. 11(26): p. 3319–31. [PubMed: 12471058]
19. Robson AG, et al. , ISCEV Standard for full-field clinical electroretinography (2022 update). Doc Ophthalmol, 2022. 144(3): p. 165–177. [PubMed: 35511377]
20. Sustar M, et al. , ISCEV extended protocol for the photopic On-Off ERG. Doc Ophthalmol, 2018. 136(3): p. 199–206. [PubMed: 29934802]
21. Robson AG, et al. , ISCEV guide to visual electrodiagnostic procedures. Doc Ophthalmol, 2018. 136(1): p. 1–26.
22. Jiang X, et al. , Prevalence of electronegative electroretinograms in a healthy adult cohort. BMJ Open Ophthalmol, 2021. 6(1): p. e000751.
23. Thompson DA, et al. , Retinal on-pathway deficit in congenital disorder of glycosylation due to phosphomannomutase deficiency. Arch Ophthalmol, 2012. 130(6): p. 712–9. [PubMed: 22801829]
24. Sieving PA, Photopic ON- and OFF-pathway abnormalities in retinal dystrophies. Trans Am Ophthalmol Soc, 1993. 91: p. 701–73. [PubMed: 8140708]
25. Tremblay F, et al. , Duchenne muscular dystrophy: negative scotopic bright-flash electroretinogram and normal dark adaptation. Can J Ophthalmol, 1994. 29(6): p. 280–3. [PubMed: 7834567]
26. Jensen H, et al. , Duchenne muscular dystrophy: negative electroretinograms and normal dark adaptation. Reappraisal of assignment of X linked incomplete congenital stationary night blindness. J Med Genet, 1995. 32(5): p. 348–51. [PubMed: 7616540]
27. Pillers DA, et al. , mdxCv3 mouse is a model for electroretinography of Duchenne/Becker muscular dystrophy. Invest Ophthalmol Vis Sci, 1995. 36(2): p. 462–6. [PubMed: 7843915]
28. Pillers DA, et al. , Duchenne/Becker muscular dystrophy: correlation of phenotype by electroretinography with sites of dystrophin mutations. Hum Genet, 1999. 105(1–2): p. 2–9. [PubMed: 10480348]
29. Ricotti V, et al. , Ocular and neurodevelopmental features of Duchenne muscular dystrophy: a signature of dystrophin function in the central nervous system. Eur J Hum Genet, 2016. 24(4): p. 562–8. [PubMed: 26081639]
30. Orlandi C, et al. , Transsynaptic Binding of Orphan Receptor GPR179 to Dystroglycan-Pikachurin Complex Is Essential for the Synaptic Organization of Photoreceptors. Cell Rep, 2018. 25(1): p. 130–145.e5. [PubMed: 30282023]

31. Haro C, et al. , Expression in retinal neurons of fukutin and FKRP, the protein products of two dystroglycanopathy-causative genes. Mol Vis, 2018. 24: p. 43–58. [PubMed: 29416295]

32. Uribe ML, et al. , Retinal Proteomics of a Mouse Model of Dystroglycanopathies Reveals Molecular Alterations in Photoreceptors. J Proteome Res, 2021. 20(6): p. 3268–3277. [PubMed: 34027671]

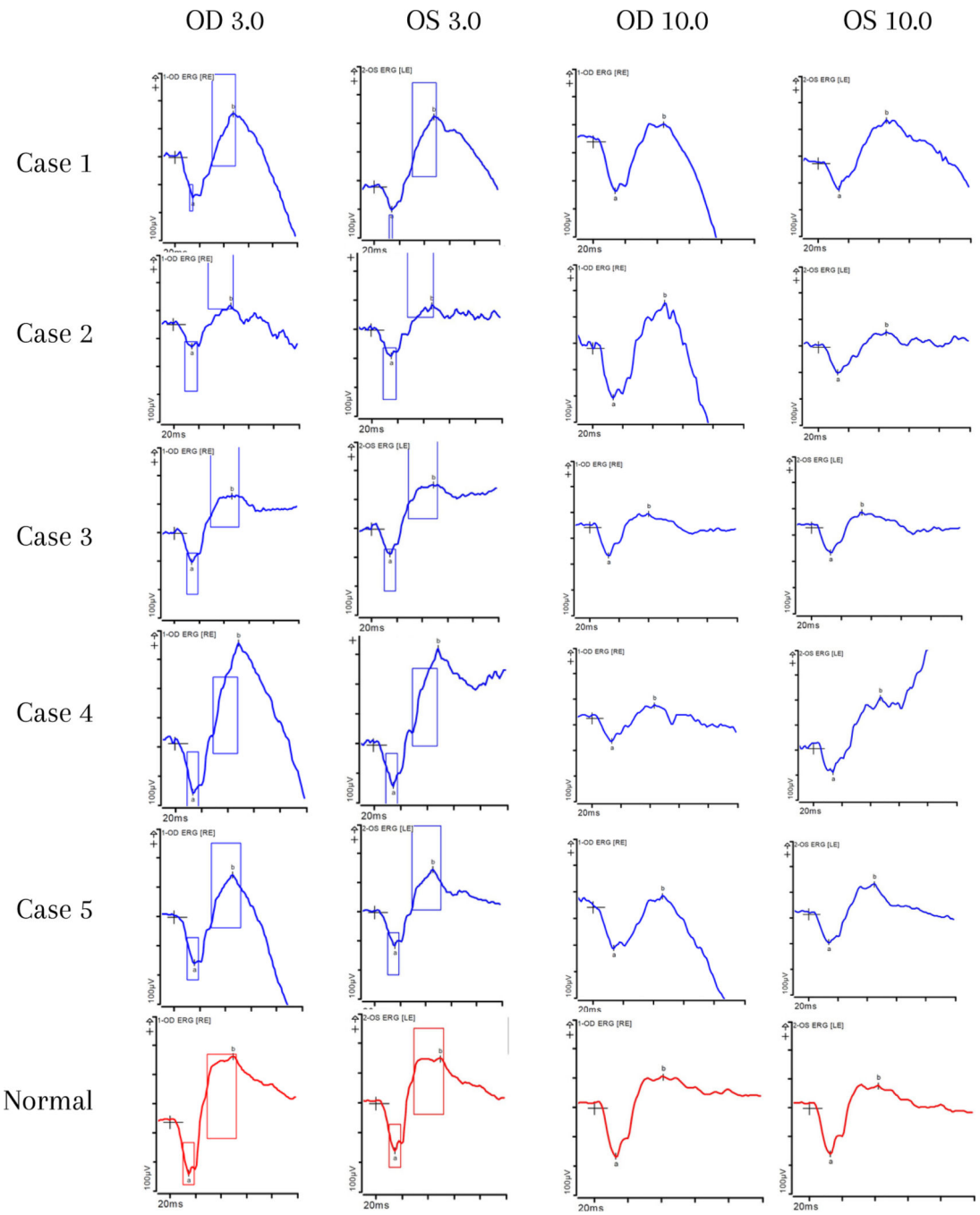

Figure 1:

Full Field electroretinogram Dark- Adapted 3.0 and 10.0 waveforms from the right and left eye for five participants with LGMDR9 in comparison to waveforms from a normal control. (OD=right eye, OS=left eye)

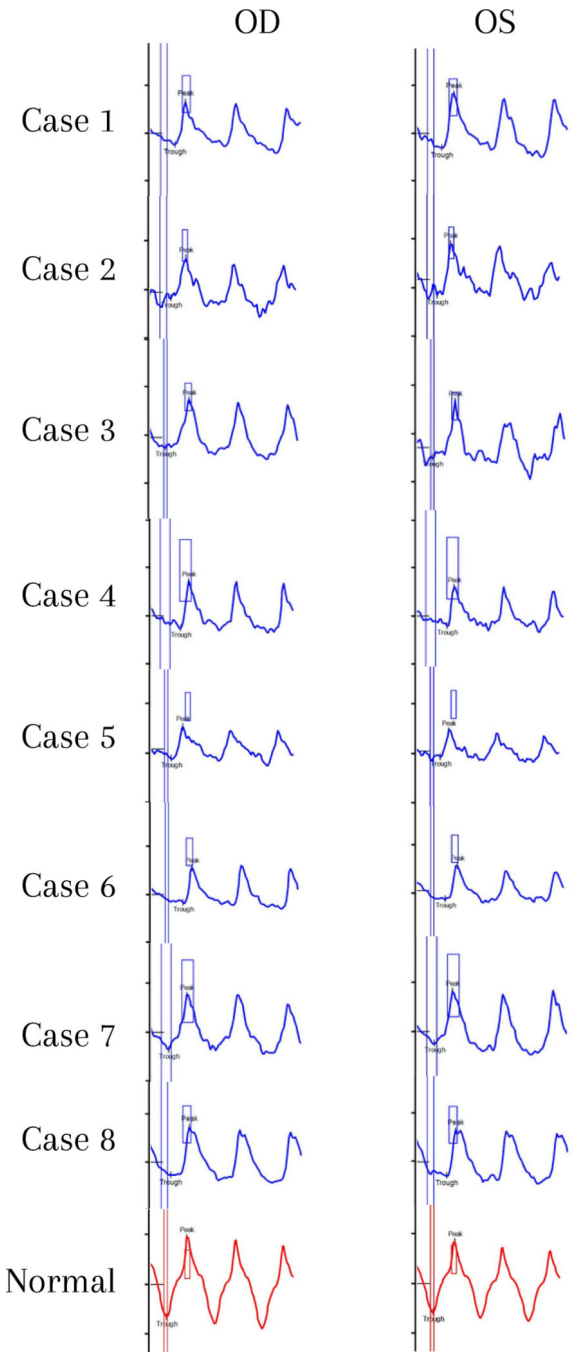

Figure 2:
Light Adapted 30 Hz flicker electroretinogram waveforms for all participants with LGMDR9 in comparison to waveforms from a normal control. (OD=right eye, OS=left eye)

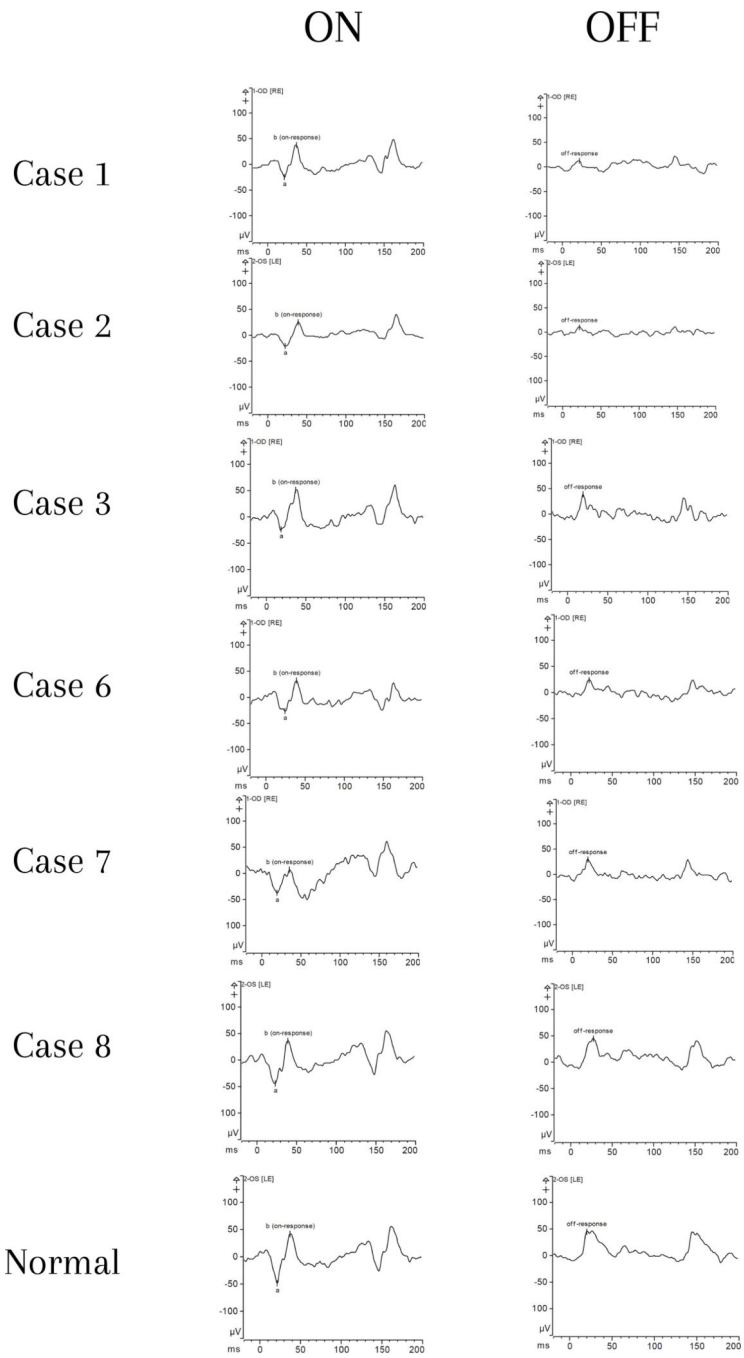

Figure 3:
Light Adapted ON and OFF response waveforms from one eye for six participants with LGMDR9 in comparison to waveforms from a normal control.

Table I:

Patient Demographics and genetic data. M/F: Male/Female,. LGMDR9: limb-girdle muscular dystrophy type 9, *FKRP*: fukutin-related protein.

STUDY ID	AGE	M/F	DIAGNOSIS	GENE	MUTATION
CASE 1	32	M	LGMDR9	*FKRP*	homozygous c.826C>A
CASE 2	30	M	LGMDR9	*FKRP*	homozygous c.826C>A
CASE 3	53	M	LGMDR9	*FKRP*	homozygous c.826C>A
CASE 4	22	M	LGMDR9	*FKRP*	c.826C>A and c.646C>T
CASE 5	56	M	LGMDR9	*FKRP*	homozygous c.826C>A
CASE 6 *	54	F	LGMDR9	*FKRP*	c.826 C>A and c.586 G>A
CASE 7 *	28	F	LGMDR9	*FKRP*	homozygous c.826C>A
CASE 8 *	49	F	LGMDR9	*FKRP*	homozygous c.826C>A

* Case 6, 7 and 8 were only able to complete light adapted electroretinogram testing, 30 Hz flicker, and ON/OFF.

Table II:

Full field electroretinogram data showing the Dark-Adapted amplitudes for the following waveforms: 3.0, 10.0, and B/A ratios.

CASE ID	3.0 OD B-WAVE	3.0 OS B-WAVE	3.0 OD A-WAVE	3.0 OS A-WAVE	3.0 OD B/A	3.0 OS B/A	10.0 OD B-WAVE	10.0 OS B-WAVE	10.0 OD A-WAVE	10.0 OS A-WAVE	10.0 OD B/A	10.0 OS B/A
CASE 1	304.1	308.9	−134.1	−96.58	2.26	3.19	247.5	257.9	−117.2	−84.05	2.11	3.06
CASE 2	164.3	206.3	−84.86	−101.4	1.93	2.03	140.6	159.4	−88.07	−96.73	1.59	1.64
CASE 3	239.2	240.6	−103.6	−92.99	2.3	2.58	160.5	162.8	−99.27	−85.29	1.61	1.9
CASE 4	543.6	486.7	−178.9	−141.1	3.03	3.44	467.9	318.74	−154.9	−96.23	3.02	3.31
CASE 5	304.4	262	−160.5	−110.2	1.89	2.30	246.4	228.6	−185.4	−126.6	1.32	1.8
AVERAGE	311.12	300.9	−132.4	−108.45	2.28	2.7	252.58	225.488	−128.9	−97.78	1.93	2.342
NL1	405.7	313.7	−178.3	−160.4	2.28	1.96	275.2	239	−166.8	−159.1	1.65	1.50
NL2	291.2	245.5	−172.3	−140.7	1.68	1.74	206	171.9	−157.9	−122.3	1.30	1.41
NL3	556.8	642.4	−305	−396.7	1.83	1.62	365.1	383.1	−272.6	−275.4	1.34	1.39
NL4	359.5	360.1	−101.1	−115.8	3.56	3.11	256.5	278.4	−118	−116.4	2.17	2.39
NL5	326.1	263.3	−134	−101.1	2.43	2.60	321.2	236.9	−162.5	−111.6	1.97	2.12
AVERAGE	387.86	365	−178.2	−182.94	2.36	2.21	284.8	261.86	−175.56	−156.96	1.69	1.76

All amplitudes listed in microvolts. An electronegative ERG has a b/a ratio <1. (OD: right eye, OS: left eye, DA: Dark-adapted).

*Case 6, 7 and 8 were only able to complete light adapted electroretinogram testing, 30 Hz flicker, and ON/OFF testing and therefore have no data to list for this table.

Table III:

Light adapted waveform amplitudes of participants and controls. All amplitudes listed in microvolts.

SUBJECTS	3.0 OD B-WAVE	3.0 OS B-WAVE	3.0 OD A-WAVE	3.0 OS A-WAVE
CASE 1	112.3	120.9	−19.13	−17.36
CASE 2	113.7	123.5	−23.64	−36.12
CASE 3	110.3	105.7	−22.87	−22.06
CASE 4	143.8	119.6	−36.52	−23.14
CASE 5	123.8	93.15	−24.71	−16.88
CASE 6	93.00	92.96	−24.57	−19.89
CASE 7	160.3	167.5	−30.81	−31.41
CASE 8	145.9	149.5	−37.65	−40.98
AVERAGE	125.38	121.60	−27.64	−25.98
NL1	182.6	150.6	−39.65	−36.59
NL2	157.7	142.1	−29.23	−23.5
NL3	235.1	240.6	−41.91	−58.13
NL4	123.9	127.8	−27.95	−27.1
NL5	119.9	96.58	−21.95	−17.2
NL11	203.7	116.9	−46.23	−24.45
NL12	124.4	118.3	−25.82	−24.01
NL13	169.9	168.1	−27.88	−27.72
AVERAGE	164.65	145.12	−32.57	−29.83

Table 4

Rapid ON/OFF testing performed using standard ISCEV protocols. All amplitudes listed in microvolts

	RAPID ON (OD)			RAPID ON (OS)			RAPID OFF	
STUDY ID	B amp	A amp	B/A	B amp	A amp	B/A	OD amp	OS amp
CASE 1	63.8	−24.18	2.63	75.58	−31.85	2.37	14.01	13.11
CASE 2	38.29	−52.48	0.73	46.9	−19.68	2.38	31.15	12.15
CASE 3	74.22	−23.22	3.19	65.13	−28.0	2.32	32.57	30.13
CASE 6	57.16	−25.17	2.67	50.81	−28.44	1.79	31.34	26.5
CASE 7	45.09	−36.51	1.23	78.43	−10.08	7.78	31.61	19.79
CASE 8	79.68	−52.45	1.51	81.79	−43.66	1.87	39.90	47.09
AVERAGE	59.70	−35.66	1.99	66.44	−26.95	3.08	30.09	24.79
NL6	66.26	−27.55	2.41	74.71	−45.9	1.62	53.32	77.46
NL7	67.34	−24.56	2.74	67.76	−26.68	2.54	19.11	21.96
NL8	100.3	−38.13	2.63	72.61	−25.23	2.88	33.17	28.41
NL9	91.01	−45.16	2.00	69.14	−34.65	1.94	70.17	51.22
NL10	85.86	−50.29	1.71	86.41	−56.95	1.52	52.82	49.17
NL11	110.2	−43.53	2.42	63.48	−20.87	3.04	54.57	21.64
AVERAGE	86.82	−38.20	2.41	72.35	−35.05	2.25	47.19	41.64