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Epidemiology and natural history in 101 subjects with FKRP-related limb-girdle muscular dystrophy R9. The Norwegian LGMDR9 cohort study (2020)

Synnøve M. Jensen[a,b,*], Kai Ivar Müller[a,b,j], Svein Ivar Mellgren[a,b], Laurence A. Bindoff[c,d,e], Magnhild Rasmussen[f,g], Kristin Ørstavik[g], Christoffer Jonsrud[h], Kristian Tveten[i], Øivind Nilssen[b,h], Marijke Van Ghelue[b,h], Kjell Arne Arntzen[a,b]

[a] National Neuromuscular Centre Norway and Department of Neurology, University Hospital of North Norway HF, Tromsø, PO Box 100, N-9038, Tromsø, Norway
[b] Department of Clinical Medicine, University of Tromsø – The Artic University of Norway, PO Box 6050 Langnes, N-9037, Tromsø, Norway
[c] Department of Clinical Medicine (K1), University of Bergen, N-5021, Bergen, Norway
[d] Department of Neurology, Haukeland University Hospital, PO Box 1400, N-5021, Bergen, Norway
[e] National Unit of Newborn Screening and Advanced Laboratory Diagnostics, Oslo University Hospital, PO Box 4950 Nydalen, N-0424, Oslo, Norway
[f] Department of Clinical Neurosciences for Children, Oslo University Hospital, PO Box 4950 Nydalen, N-0424, Oslo, Norway
[g] Unit for Congenital and Hereditary Neuromuscular Conditions (EMAN), Department of Neurology, Oslo University Hospital, PO Box 4950 Nydalen, N-0424, Oslo, Norway
[h] Department of Medical Genetics, Division of Child and Adolescent Health, University Hospital of North Norway HF, PO Box 55, N-9038, Tromsø, Norway
[i] Department of Medical Genetics, Telemark Hospital Trust, PO Box 2900 Kjørbekk, N-3710, Skien, Norway
[j] Department of Neurology, Hospital of Southern Norway, PO box 416 Lundsiden, 4604, Kristiansand S, Norway

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ABSTRACT

We aimed to investigate the epidemiology and natural history of FKRP-related limb-girdle muscular dystrophy R9 (LGMDR9) in Norway. We identified 153 genetically confirmed subjects making the overall prevalence 2.84/100,000, the highest reported figure worldwide. Of the 153 subjects, 134 (88 %) were homozygous for *FKRP* c.826C>A giving a carrier frequency for this variant of 1/101 in Norway. Clinical questionnaires and patient notes from 101 subjects, including 88 c.826C>A homozygotes, were reviewed, and 43/101 subjects examined clinically. Age of onset in c.826C>A homozygotes demonstrated a bimodal distribution. Female subjects showed an increased cumulative probability of wheelchair dependency and need for ventilatory support. Across the cohort, the need for ventilatory support preceded wheelchair dependency in one third of the cases, usually due to sleep apnea. In c.826C>A homozygotes, occurrence of cardiomyopathy correlated positively with male gender but not with age or disease stage. This study highlights novel gender differences in both loss of ambulation, need for ventilatory support and the development of cardiomyopathy. Our results confirm the need for vigilance in order to detect respiratory insufficiency and cardiac involvement, but indicate that these events affect males and females differently.

1. Introduction

The limb-girdle muscular dystrophies (LGMDs) comprise a group of inherited muscle diseases characterized by progressive proximal weakness and muscle wasting [1]. The recessive LGMDR9, formerly known as LGMD2I [2], is caused by pathogenic variants in the *FKRP* gene identified in 2001 [3]. In addition to limb girdle muscular dystrophy, FKRP-related diseases also include the very rare congenital muscular dystrophy type 1C (MDC1C), muscle-eye-brain disease (MEB) and Walker-Warburg syndrome (WWS) [4,5]. Fukutin Related Protein (FKRP) is a glycosyl transferase involved in the post-translational *O*-glycosylation of the sarcolemma protein α-dystroglycan and the *N*-glycosylation of the extracellular matrix (ECM) protein fibronectin [4,6-8] in the Golgi.

LGMDR9 is most frequent in North-European populations due to the distribution of the c.826C>A (p. Leu276Ile) variant. Analyses of neighbouring single nucleotide polymorphisms, performed on

* Corresponding author at: National Neuromuscular Centre Norway and Department of Neurology, University Hospital of North Norway HF (UNN), PO Box 100, N-9038, Tromsø, Norway.
E-mail address: synnove.magnhild.jensen@unn.no (S.M. Jensen).

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families with *FKRP* c.826C>A, indicate that this variant has a common ancestral origin (founder) rather than being a mutational hot spot with recurrent events [9,10]. The allele frequencies of the c.826C>A variant differ in various populations: in the Swedish population it is 0.0046 (carrier frequency 1/109), in North-Western Europeans 0.0023 (carrier frequency 1/218), in Finns 0.0011 (carrier frequency 1/469) and in African/African-Americans < 0.00034 (carrier frequency 1/1490) [11]. The allele has not been detected in the Asian population [11] who have a different spectrum of *FKRP* disease-causing variants [12–14]. The highest prevalence of LGMDR9 has been recorded in Norway: in 2009 the national prevalence was estimated to be 1.85/100,000 [15]. More recently the prevalence was found to be 5.88/100,000 in Northern Norway [16].

Natural history studies of LGMDR9 have mostly been based on limited sample-size although studies with 32-69 subjects have been reported in: Norway [15], USA [17,18], UK [19] and Denmark [20,21]. A global patient registry containing > 300 registered subjects has also been established [22]. LGMDR9 manifests with a wide clinical variability and evidence suggests that this in part reflects the genotype: subjects homozygous for c.826C>A express a milder phenotype compared to compound heterozygous subjects [15,19,20,22]. Data from the global FKRP registry also showed a later onset (19 ± 12 versus 7 ± 7 years), a lower proportion of wheelchair dependency (18 % versus 40 %), and a later need for ventilatory support (47 ± 14 versus 33 ± 13 years) [22]. Cardiac studies predicted that c.826C>A homozygotes have a 50 % probability of cardiomyopathy by age 50 versus 20 years in c.826C>A compound heterozygotes [17,19]. Other studies found considerable variation in clinical severity within c.826C>A homozygotes [10,23,24] with age of onset ranging from 0-50 years [15] and variable age at loss of ambulation [22].

In the present study, we investigated the prevalence, the spectrum of variants and the clinical patterns of LGMDR9 in Norway. We searched for possible predictors for developing wheelchair dependency, need for ventilatory support and cardiomyopathy. Our goal was to provide data that may be useful for improving clinical practice, for clinical trials, and translational research.

2. Materials and methods

2.1. Inclusion criteria

All living subjects with genetically confirmed LGMDR9 residing in Norway were included in the epidemiological part of the study. The subjects were identified through diagnostic patient registries at: The Medical Genetics Departments of the University Hospital of North Norway HF (UNN) and Telemark Hospital Trust (THT), the Department of Neurology at Haukeland University Hospital (HUS), the Norwegian Registry of Hereditary and Congenital Neuromuscular Disorders, and by the Global FKRP Registry. All were invited to participate in the observational study. Subjects ≥ 16 years of age were also invited to participate in the baseline examination of The Norwegian LGMDR9 cohort study at the National Neuromuscular Centre Norway, UNN.

2.2. Prevalence and genetic data

The minimum overall prevalences of LGMDR9 in Norway on January 1st 2021 was calculated nationally and for each county, and separately for the paediatric and adult population. Minimum *FKRP* c.826C>A (NM_024301.5) allele- and carrier frequencies were also calculated. The population number was obtained from Statistics Norway [25]. Genotypes were recorded, and novel variants were registered in the ClinVar database [26]. The presence of the single nucleotide polymorphism, *FKRP* c.135C>T, previously found to be in linkage disequilibrium with c.826C>A, was also assessed in the Norwegian population of *FKRP* c.826C>A homozygotes [9,10]. No other FKRP-related phenotypes than LGMD were identified at the four relevant departments: The Medical Genetic Departments of UNN, HUS, THT and Oslo University Hospital.

2.3. Clinical data

Data from patient notes and questionnaires as well as clinical data from those participating in The Norwegian LGMDR9 cohort study were registered. Participants were divided by genotype into c.826C>A homozygotes and **non**-c.826C>A homozygous groups. When analysing the natural history, gender was accounted for in both groups. Symptom onset was defined as the first symptoms or clinical signs of muscle disease including myoglobinuria, but not an incidental finding of elevated serum creatine kinase (CK) or transaminases (ALAT, ASAT). Achieved independent walking was used as the criterion to differentiate LGMDR9 from congenital muscular dystrophy type 1C. Ambulation was participant reported and recorded as wheelchair dependent or not and graded according to the maximum walking distance on flat ground, the ability to climb stairs one floor, and the ability to run (Sect. 2.4). Ventilation was evaluated based on the need for ventilatory support while cardiomyopathy was assessed based on the results of routine echocardiography. The lower normal limit of left ventricular ejection fraction (LVEF) was set to 50 % in accordance with the reports.

2.4. Statistical analyses

Study data were collected and managed using REDCap[1] electronic data capture tools hosted at UNN [27,28]. Statistical analyses were performed using IBM SPSS Statistics for Windows (Version 27.0. Armonk, NY: IBM Corp.). Wilson score was employed to estimate the confidence intervals (CIs) of the lowest and highest county prevalence for comparison. The carrier frequency of the common c.826C>A variant in the population was calculated with the Hardy-Weinberg equation. Normally distributed continuous variables were described using the means and standard deviations, and non-parametric using medians and inter-quartile ranges (IQRs). Categorical variables were described as percentages. Pearson Chi-square test and Fisherś exact test with mid-p correction, as appropriate, were used to assess associations between categorical variables. Mann-Whitney U-test was used to assess the differences in continuous non-parametric distributed variables between two groups. Two-step cluster analysis was applied to assess the distribution of age at onset of the disease. For clinical endpoints with censored observations, Kaplan-Meier survival curves were estimated in specific subgroups using age as time scale. The events defined were wheelchair dependency, commence of ventilatory support, and first abnormal echocardiography, respectively. Negative subjects concerning wheelchair and ventilatory support were censored at the end of 2020, while for cardiomyopathy at last echocardiography, or at ischemic incidence (one participant). Log-rank test was used to compare survival curves between genders of similar genotype, while Cox regression between genotype groups to control

[1] REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support data capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources.

for gender. Cox proportional Hazard regression models were applied to estimate hazard ratios (HRs). Fifty percent accumulated probabilities were estimated when applicable. Ambulation was both evaluated by wheelchair dependency as a binary variable, and by a 10-point (0-9) motor composite score (MCS). MCS was composed from the questionnaire consisting of three items: walking distance on flat ground (0: unrestricted, 1: > 1000 meters, 2: > 500 meters, 3: > 50 meters, 4: > a few steps, 5: zero or a few steps); ability to climb stairs one floor independently (0 = yes, 1 = manage with stair railing, 2 = not able); ability to run (0 = no problems, 1 = yes, but not fast, 2 = not able). (Cronbachś α = 0.73, corrected item-total correlation: 1. Item: 0.73, 2. Item: 0.80, 3. Item: 0.64). It was complemented with regression analyses of cross-sectional data in order to assess potential predictors and confounding effect. MCS as dependent continuous variable was assessed by linear regression. Multivariate binomial logistic regression with maximum-likelihood method was employed to estimate odds ratios (ORs) for cardiomyopathy, need for ventilatory support and wheelchair dependency as binary dependent variables. The independent variables assessed were age, gender, age at symptom onset, symptom duration, MCS for cardiomyopathy and ventilatory support, and ventilatory support for cardiomyopathy. Following simple regression analyses, the most relevant independent variables were selected for the multivariate analyses. The significance level was set to 0.05. The Holm-Bonferroni method was applied due to multiple subgroup comparisons of the natural history (12 tests): age of onset and cumulative probability comparisons between both genotype and gender subgroups, and gender as an independent variable in the multivariate analyses. Due to the exploratory nature of the study, empirical p-values were reported while the multiple comparison test (MCT) was used to confirm or not confirm significant p-values. Results with and without correction for multiple comparisons are discussed.

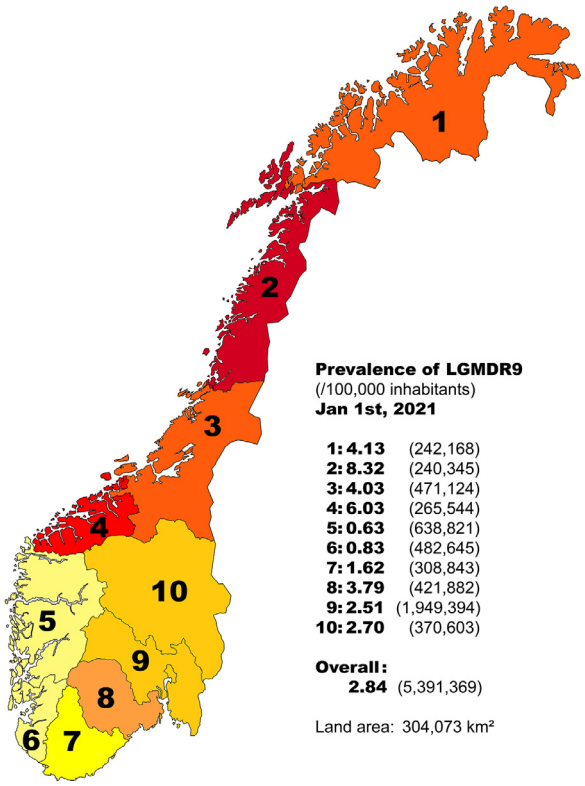

Fig. 1. Map of Norway showing minimum LGMDR9 prevalences and population sizes on national and county level as of 1st of January 2021. Oslo was merged with Viken county due to the small area and similar prevalence (number 9). Colour intensity correlates with the whole number of cases with LGMDR9 per 100,000 population. Sources: Map from Wikimedia common (with adaptation), numbers from Statistics Norway.

2.5. Approval and patient consent

All participants provided informed written consent for the collection and use of clinical data. The study was approved by the Ethical Review Board of North Norway (2018/1968/REK nord), and by the Data Protection Officer at UNN.

3. Results

3.1. Prevalence and molecular data

On January 1st, 2021, Norwayś population was 5,391,369. We identified 153 living subjects with genetically confirmed LGMDR9 giving a minimum point prevalence of 2.84/100,000 for the whole population. This could be further subdivided into 1.98/100,000 in the paediatric (< 18 years), and 3.06/100,000 in the adult population.

Prevalence was highest in the northern and central parts of Norway (counties 1-4, Fig. 1). The individual county prevalences ranged from 0.63/100,000 (CI: 0.24, 1.61) in the southwest to 8.32/100,000 (CI: 5.39, 12.85) in Nordland county in the north (county 5 and 2, Fig. 1). There was also regional clustering of LGMDR9 subjects within counties (not shown).

Of the total 153 LGMDR9 subjects, 134 (87.6 %) were *FKRP* c.826C>A homozygotes (52 % females), 16 (10.4 %) carried the c.826C>A on one allele, while three subjects (2.0 %) did not have the c.826C>A variant. The total carrier frequency was calculated to 1/94 individuals, and the c.826C>A carrier frequency to 1/101. Two novel *FKRP* variants were identified: c.141_151del11 p.(Arg48Profs*9) and c.166T>A p.(Phe56Ile) (Table 1). These have not been reported in the literature and are not present in the gnomAD population database [11]. The *FKRP* c.141_151del11 variant results in an out of frame deletion early in the coding exon 4, with a predicted consequence on the protein p.(Arg48Profs*9). This is a null allele causing FKRP truncation. Accordingly, the patient who is compound heterozygous for both this variant and c.826C>A most probably exclusively expresses the latter variant causing the LGMDR9 phenotype (Table A1). We also found a subject who was compound heterozygous for *FKRP* c.166T>A and c.826C>A (Table A1). We concluded that the c.166T>A variant was likely pathogenic based on the recommendations of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (criteria PM2, PM3, PP2 og PP4) [29]. The patient manifests an LGMD phenotype with limb-girdle weakness, calf pseudohypertrophy, scapular winging, hyperCKemia and dilated cardiomyopathy. Symptoms began with post exercise myalgia at age 13 years. Histological features support a diagnosis of primary structural muscle disease but there was insufficient material for additional staining including dystroglycan. EMG supports chronic myopathy. Muscle imaging has not been performed. The PP4-criterion was considered fulfilled when tests of genes with overlapping phenotypes disclosed no other findings. This patient was tested for variants using a neuromuscular NGS panel (328 genes) and MLPA of dystrophin and alpha-, beta-, delta and gamma-sarcoglycan. The *FKRP* c.135C>T/c.135C>T genotype was detected in all c.826C>A homozygotes who were genetically confirmed at UNN (n = 127). For comparison, the frequency of the c.135C>T allele among Europeans (non-Finnish) is reported to be 0.14 and the homozygote frequency observed in the same population is 0.01 [11].

Table 1
FKRP Variants (NM_024301.5) identified in the Norwegian population of LGMDR9

N subjects (N fam)	Variant on FKRP allele one	Effect on FKRP protein	FKRP variant in allele two	Effect on FKRP protein
2 (1)	c.160C>T	p.(Arg54Trp)	c.160C>T	p.(Arg54Trp)
134 (107)	c.826C>A	p.(Leu276Ile)	c.826C>A	p.(Leu276Ile)
1 (1)	c.826C>A	p.(Leu276Ile)	c.141_151del11†	p.(Arg48Profs*9)
1 (1)	c.826C>A	p.(Leu276Ile)	c.166T>A†	p.(Phe56Ile)
1 (1)	c.826C>A	p.(Leu276Ile)	c.328C>T	p.(Arg110Trp)
1 (1)	c.826C>A	p.(Leu276Ile)	c.469G>C	p.(Ala157Pro)
3 (3)	c.826C>A	p.(Leu276Ile)	c.899T>C	p.(Val300Ala)
1 (1)	c.170_189del20‡	p.(Glu57Alafs*68)	c.899T>C	p.(Val300Ala)
7 (5)	c.826C>A	p.(Leu276Ile)	c.962C>A	p.(Ala321Glu)
2 (2)	c.826C>A	p.(Leu276Ile)	c.1323T>G	p.(Phe441Leu)

†Novel variants
‡Not registered in genetic database, but reported in a previous Norwegian study (Stensland et al, 2011) [15].

3.2. Clinical data

3.2.1. Participants

Of the 153 LGMDR9 individuals identified, 101 (66.0 %) participated in the observational study. Patient notes and questionnaires were obtained from all participants, and 43/101 (42.6 %) were also examined clinically. Of 101 participants, 88 were c.826C>A homozygotes. Thirteen participants harboured other genotypes and included 12 c.826C>A compound heterozygotes and one who was homozygous for c.160C>T (Table A1). The average age of c.826C>A homozygous participants was 43 ± 18 years compared to 31 ± 17 years ($p = 0.037$) for those with other genotypes. Symptom duration was 30 ± 19 and 23 ± 14 years, respectively ($p = 0.22$). It is likely that there is an overlap of participants between the current study and the two previous Norwegian studies [15,30], and the global registry [22].

3.2.2. Clinical characteristics

The clinical characteristics are summarized in Table 2 (demographics and natural history) and Table 3 (clinical features and symptoms). All participants achieved independent walking. In three subjects presenting after the age of one year, onset could be traced back to abnormalities in gross motor development in the first year of life. One was a c.826C>A homozygote examined aged 10 years for abdominal pain and elevated CK and transaminases. It was found that she had difficulty sitting up. She had been late in sitting and did not roll or crawl independently. Independent walking was achieved at normal age, 15 months. The other two patients had other genotypes (Table A1, Subjects 1 and 3): despite their diagnostic work-up being at 3 and 6 years, signs were observed at 9 months (specifics missing) and 7 months (hypotonia) respectively, and the latter had delayed independent walking.

The most common initial symptoms were lower limb weakness and exertional intolerance including myalgia, stiffness or cramps (Table 3). Three subjects had a relatively sudden onset of symptoms. One c.826C>A homozygote who developed hip pain and lower limb weakness (CK unknown) at the age of 10 was initially considered to have a viral arthritis although no infection was confirmed. Moreover, the gait difficulties persisted. Seventeen years later, she developed ankle arthritis and was diagnosed with sarcoidosis. The diagnosis of LGMDR9 was made some years later. Muscle biopsy was compatible with LGMDR9 including lack of α-dystroglycan expression. Another c.826C>A homozygote developed head drop and lower limb weakness aged 20 months following a fever. CK was 2,300 U/L. No infection was found and the lower limb weakness persisted. Similarly, a c.826C>A compound heterozygote (Table A1, Subject 10) had at the age of 3 years fever-related myoglobinuria and 4 days later hypotonia involving neck, truncal and lower limb muscles, a CK 18,000 U/L and no signs of infection. It was not reported whether there was full recovery or not.

CK values at diagnostic work-up were reported in 76/88 c.826C>A homozygotes and 12/13 subjects in the **non**-c.826C>A homozygous group. The median peak CK value was 6,000 U/L and 9,000 U/L, respectively, and the range between 206 and 42,000 U/L. One c.826C>A homozygote participant in her early 40s with fifteen years of slowly progressive lower limb weakness did not have elevated CK values. In 18/100 participants, investigation for a muscle disorder was prompted by the finding of an elevated CK or transaminases detected during work-up for non-muscular complaints, most commonly abdominal pain (10/18). In 15 of these, signs or symptoms of muscle disease were present at initial examination. In four males (three c.826C>A homozygotes), diagnostic investigations occurred secondary to cardiac failure at the age of 20-35 years. Three of them had concomitant hyperCKemia (CK 3,000-10,000 U/L) (missing data on the fourth) and three had preceding muscle symptoms.

The most frequently reported clinical features in both genotype groups were proximal weakness, calf pseudohypertrophy, mild scapular winging, and tendo-achilles contractures. Additionally, scoliosis and multiple contractures were relatively frequent in the **non**-c.826C>A homozygous group (Table 3). In this group there was also one subject with a thoracic kyphosis and another with a rigid spine. Plain X-rays of the spine were usually not performed. The scoliosis was in several cases specified in the patient notes as mild and only in two cases as pronounced. The frequency of self-reported scoliosis was 11/101. Of these 11 subjects, five had received no treatment, five physiotherapy and one both physiotherapy and corset. None had undergone scoliosis surgery.

Oropharyngeal symptoms were reported by 20 participants (Table 3) of whom 11 had initiated ventilatory support. Dysarthria usually coexisted with dysphagia and both occurred at a median age of 40 years. Four subjects specified their difficulties as swallowing wrongly, and two as food getting stuck in the throat. Dysarthria was described as slurred speech by two subjects. One subject with difficulties chewing also required ventilation both night and day as well as percutaneous endoscopic gastrostomy (PEG). One c.826C>A homozygote and three **non**-c.826C>A homozygous subjects had PEG, all related to breathing difficulties including two subjects who needed it only temporarily. Two c.826C>A homozygous females reported cramps in the tongue and/ or throat. One subject was 24 years old with exertional myalgia from the age of 6 years. She described cramps in the tongue and throat from the age of 10 years as well as a tendency for food to get stuck in her throat and having a slurred speech. Respiratory difficulties were, however, not reported. The second subject was a 51-year-old female with obstructive sleep apnea (OSA) who reported painful cramps in tongue or throat when

Table 2
Demographic and natural history data of the LGMDR9 cohort (N = 101)

FKRP genotype	**c.826C>A / c.826C>A**			**Other**		
Gender	**Total**	**Females**	**Males**	**Total**	**Females**	**Males**
N (%)	88/88 (100)	43/88 (48.9)	45/88 (51.1)	13/13 (100)	8/13 (61.5)	5/13 (38.5)
Age (years) Mean ± SD [range]	43 ± 18 [8–78]	41 ± 19	44 ± 18	31 ± 17 [9–66]	23 ± 11	44 ± 19
Onset age (years) Median (IQR) [range]	8.5 (5-19) [0–45]	8 (6-20)	9 (5-20)	3 (1-12) [0–43]	2 (0-10)	7 (1.5-28)
W/C n (%)	25/88 (28.4)	18/43 (41.9)	7/45 (15.6)	10/13 (76.9)	7/8 (87.5)	3/5 (60.0)
Age at W/C (years) Median (IQR) [range]	36.5 (30-50) [15–60]	35.5 (31-49)	39 (22-50)	13 (9-24) [3–60]	11 (9-12)	11 (-)
Years onset to W/C Median (IQR) [range]	29 (15-36) [5–49]	27.5 (17-36)	31 (13-42)	11 (8-13) [2–27]	14 (9-23)	12 (-)
NIV initiated n (%)	27/88 (30.7)	19/43 (44.2)	8/45 (17.8)	5/13 (38.5)	2/8 (25.0)	3/5 (60.0)
Discontinued	4/88 (4.5)			0/13 (0)		
Nocturnal	20/88 (22.7)			1/13 (7.7)		
Intermittently	3/88 (3.4)			2/13 (15.4)		
Continuously	0/88 (0)			2/13 (15.4)		
Age at NIV (years) Median (IQR) [range]	45.5 (39-56) [19–71] (N.R.: 1)	46 (43-56) (N.R.: 1)	38.5 (31-62)	27 (16-47) [8–63]	17.5	31 (-)
Years W/C to NIV Median (IQR) [range]	10.0 (3-16) [-15-38] (n = 18) (N.R.: 1)	12 (6-17) (n = 13) (N.R.: 1)	0 (-9-12) (n = 5)	13.0 (4-18) [3-19] (n = 5)	9 (-) (n = 2)	16 (-) (n = 3)
CM n (%)	26/84 (31.0)	7/41 (17.1) (N.A.: 2)	19/43 (44.2) (N.A.: 2)	5/13 (38.5)	3/8 (37.5)	2/5 (40.0)
LVEF < 50 %	(N.A.: 4)			5/5 (100)		
Dilatation + borderline	20/26 (76.9)			(1+0)/5 (20.0)		
Other	(13 + 7)/26 (76.9)			0/5 (0)		
	2 [a] /26 (7.7)					
Age at CM (years) Median (IQR) [range]	36.5 (26-47) [13–70]	39 (28-47)	35 (26-47)	24 (19-30) [18–31]	28 (-)	19 (-)
Arrhythmia n (%)	12 [b] /88 (13.6)	5/43 (11.6)	7/45 (15.6)	0/13 (0)	0/8 (0)	0/5 (0)
Cardiac medication n (%)	19/87 (21.8)	4/43 (9.3)	15/44 (34.1)	4/13 (30.8)	2/8 (25.0)	2/5 (40.0)
ACEI/α2-blocker + 0	N.R.: 1		N.R.: 1	3/4 (75.0)		
ACEI/α2-blocker + 1	13/19 (68.4)			1/4 (25.0)		
ACEI/α2-blocker + ≥ 2	3/19 (15.8)			0/4 (0)		
	3 /19 (15.8)					
Cardiac electrical implant n; at age (years)	3	0	ICD: 1; 38 CRT-D: 2; 43, 50	0	0	0
Cardiac transplant n; at age (years)	1	0	1; 57	0	0	0

IQR = interquartile range, N.R = not reported, N.A.: not assessed, W/C = wheelchair dependency, NIV = non-invasive ventilatory support, CM = assumed LGMDR9-related cardiomyopathy, LVEF = left ventricular ejection fraction, ICD = Implantable Cardiac Defibrillator, CRT-D = Cardiac Resynchronization Therapy-Device
[a]Delayed relaxation (no comorbidity), mixed hypertrophy and dilatation (concomitant hypertension)
[b]Electrical disturbances: Sinus bradycardia (2 males), atrial flimmer/flutter (1 female, 2 males), frequent ventricular extrasystoles (1 female, 1 male), ventricular tachycardia (1 male), ventricular dyssynchrony/ left bundle branch block (3 males), right bundle branch block (1 male), supraventricular extrasystoles/palpitations (3 females)
[c]Cardiac involvement with cardiac medication: Angiotensin-Converting Enzyme Inhibitor (ACEI), α2-blocker, β-blocker, amiodarone, spironolactone, warfarin

yawning. The cramps caused difficulties with swallowing and could last for hours or days. She also had troublesome cramps in the extremities triggered by minor physical exertion, sudden movement or trauma. It is unknown whether the cramps have been investigated further.

Wheelchair dependency was present in 35/101 participants, and non-invasive ventilatory support initiated in 32/101. The indication for ventilatory support was extrapulmonary restrictiveness in 15/32, OSA in 7/32, and both combined in 10/32. In two c.826C>A homozygotes age 52 and 68 years, ventilatory support was instituted after acute respiratory failure with hypercapnia. Both were wheelchair dependent. In 18/101 participants, pulmonary investigations were not performed, and it was uncertain in another three. None of these 21 subjects reported dyspnea.

Cardiomyopathy with no other identified cause was recognized in 31/97 (32.0 %) participants. In 10 subjects, abnormalities were noticed on the first echocardiography. Four c.826C>A homozygous males had a severe cardiomyopathy: three with an implanted electronic device, and one who required cardiac transplantation. Conduction abnormalities or arrhythmias were reported in nine c.826C>A homozygotes with cardiomyopathy, and palpitations or supraventricular extrasystoli in three c.826C>A homozygous females without cardiomyopathy (Table 2). The earliest age at which cardiomyopathy was detected was in a 13-year-old c.826C>A homozygous boy with fractional shortening. At 17 years of age he developed a dilated cardiomyopathy with LVEF 50-55 % and MRI showing patchy fibrosis. Cardioactive medication was then initiated. One c.826C>A homozygous male, with assumed LGMDR9-related dilated cardiomyopathy and cardiac implant (CRT-D), had concomitant pulmonary sarcoidosis, which could potentially have been the underlying cause. MRI showed fibrosis subepicardial in the lateral wall and septum that was considered atypical for sarcoidosis. Another c.826C>A homozygous male with exertional stiffness in the thighs from the age of 12 years had, at the age of 14, increasing exertional stiffness, dyspnea and nausea and was diagnosed with rhabdomyolysis (CK > 20,000 U/L) and dilated cardiomyopathy. There was intramural contrast enhancement on MRI. Coronary CT scan and virus tests were initially negative. MRI one year later showed similar findings and virus testing was positive for respiratory syncytial virus, which

Table 3
Clinical features of 101 LGMDR9 participants

***FKRP* genotype**	**c.826C>A / c.826C>A (n = 88)**	**Other (n = 13)**
Onset symptom(s) n (%)		
Lower limb weakness	51/88 (58.0)	4/13 (30.8)
Exertional pain, stiffness or cramps	33/88 (37.5)	2/13 (15.4)
Exertional fatigue	9/88 (10.2)	2/13 (15.4)
Exertion-induced myoglobinuria	8/88 (9.1)	0/13 (0)
Upper limb weakness	5/88 (5.7)	1/13 (7.7)
Delayed motor development	4/88 (4.5)	2/13 (15.4)
Toe walking	2/88 (2.3)	1/13 (7.7)
General fatigue	2/88 (2.3)	1/13 (7.7)
Symptomatic cardiac failure	1/88 (1.1)	0/13 (0)
Myoglobinuria n (%)	28/88 (31.8) N.D.: 10	3/13 (23.1) N.D.: 3
Hypertrophy		
Calves	53/88 (60.2) N.D.: 18	10/13 (76.9) N.D.: 2
Other[a]	16/88 (18.2) N.D.: 40	1/13 (7.7) N.D.: 8
Scapular winging n (%)	41/88 (46.6) N.D.: 8	9/13 (69.2) N.D.: 4
Contractures n (%)		
Tendo-achilles	32/88 (36.4) N.D.: 22 Tenotomy: n = 0	8/13 (61.5) N.D.: 1 Tenotomy: n = 1
Other[b]	4/88 (4.5) N.D.: 37	4/13 (30.8) N.D.: 3
Multiple lower limb	3/88 (3.4)	1/13 (7.7)
Multiple lower + upper limbs	1/88 (1.1)	3/13 (23.1)
Scoliosis n (%)	16/88 (18.2)	7/13 (53.8)
Oropharyngeal symptoms n (%)	15/88 (17.0)	5/13 (38.5)
	Dysphagia 9/88 (10.2)	Dysphagia 5/13 (38.5)
	Dysarthria 9/88 (10.2)	Dysarthria 1/13 (7.7)
	Tongue/ throat cramps 2/88 (2.2)	Jaw fatigue 1/13 (7.7)
		Chewing difficulties 1/13 (7.7)
Macroglossia n (%)	9/88 (10.2) N.D.: 48	2/13 (15.4) N.D.: 9
Facial weakness n (%)	6/88 (6.8) N.D.: 19	2 /13 (15.4) N.D.: 1

The numbers in brackets indicate the percentage of participants with positive findings within each genotype subgroup. N.D. = not described
[a]Pseudohypertrophy, other: thighs (quadriceps n = 10, unspecified n = 3), forearms (n = 3), trapezius (n = 2), gluteus (n = 1)
[b]Contractures, other: elbows (n = 4), knees (n = 3), hips (n = 3), fingers (n = 1)

is usually benign in this age group. He remained asymptomatic. Two years later, echocardiography showed normal findings despite taking no cardioactive medication. One c.826C>A homozygous male had dilated cardiomyopathy which was assumed to be post ischemic and was not included.

Several participants had cardiovascular disease or risk factors. Myocardial infarction was reported in four males (8.0 %) and detected subclinically on scintigraphy in one female (2.0 %). One additional male and female also had coronary artery disease. Obesity was reported in five males (10.0 %) and 11 females (21.6 %). Hypertension was reported in 12 males (24.0 %) and four females (7.8 %), elevated cholesterol in four males (8.0 %) and two females (3.9 %) and diabetes mellitus in three males (6.0 %) and one female (2.0 %). Three males (6.0 %) and three females (5.9 %) were habitual smokers, and 12 males (24.0 %) and eight females (15.7 %) were former smokers.

Four more subjects had cardiac MRI. The MRI findings were consistent with the echocardiography findings showing dilatation and/ or reduced contractility. Additionally, one subject showed fibrosis in the lateral wall, whereas in another subject apical fibrosis was reported.

3.2.3. Natural history of the FKRP c.826C>A homozygous cohort

The natural history data are summarized in Table 2. In c.826C>A homozygotes, median age of symptom onset was 8.5 years. Interestingly, a two-step cluster analysis suggested a bimodal distribution with a predominant (75 %) early-onset subgroup (mean 7 years, range 0-17) and a late-onset subgroup (mean 29 years, range 20-45) (Fig. 2) irrespective of gender (p = 0.52) (Fig. B.1). Diagnostic work-up started at a median age of 21 years (IQR 13-32). Median time from symptom onset to work-up was 10.5 years (IQR 4-20) excluding 16 subjects who had an incidental finding of elevated CK or transaminases and 14 with a previously diagnosed sibling.

Survival analysis demonstrated lower cumulative probability of wheelchair dependency in males (HR 0.22 (CI: 0.09, 0.54), p < 0.001). Females reached a 50 % probability of wheelchair dependency by 49 years (CI: 37, 61). The male survival curve did not cross the 50 % level (Fig. 3A). Regression analysis showed that MCS correlated with female gender and disease duration (Table B1) while age of onset showed no significant correlation (p = 0.23). Wheelchair dependency was equally correlated with female gender, and disease duration was borderline significant (Table B2). Age of onset showed no significant correlation (p = 0.25). MCT confirmed the gender differences.

Median age of initiation of ventilatory support (46 years) was nearly a decade later than the median age of wheelchair dependency (Fig. 4). Males showed lower accumulated probability of requiring ventilatory support (HR 0.26 (CI: 0.11, 0.65), p = 0.002). MCT confirmed the gender difference. Females reached a 50 % probability of needing ventilatory support by the age of 52 years (CI: 44, 60), whereas males, in the age range of our cohort, did not reach the 50 % level (Fig. 3B). Ventilatory support was initiated in 10/26 cases in an ambulatory stage and 7/10 due to OSA. Multivariate regression analysis showed a positive correlation with age (p = 0.0011) and MCS (p = 0.0003) but not with gender (Table B3).

Age of cardiomyopathy detection was similar to that for becoming wheelchair dependent (Fig. 4). In contrast to wheelchair dependency and ventilatory support, males had a higher cumulative probability of cardiomyopathy development (HR 2.71 (CI: 1.14, 6.46), p = 0.019). The predicted 50 % probability of cardiomyopathy development occurred at age 55 (CI: 44, 66) in males while the female survival curve did not cross the 50 % level (Fig. 3C). In the regression analysis, gender was the only significant predictor variable for cardiomyopathy. Both age and MCS were non-significant (Table B4). Accordingly, we observed that the four most severely affected cardiac patients were males

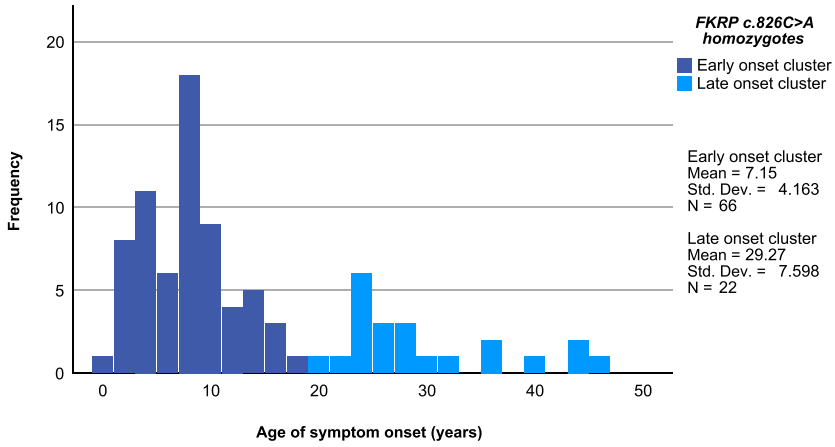

Fig. 2. Histogram demonstrating the distribution of age at onset in the FKRP c.826C>A homozygous participants (n = 88). Subjects are divided in an early and late onset group according to a two-step cluster analysis.

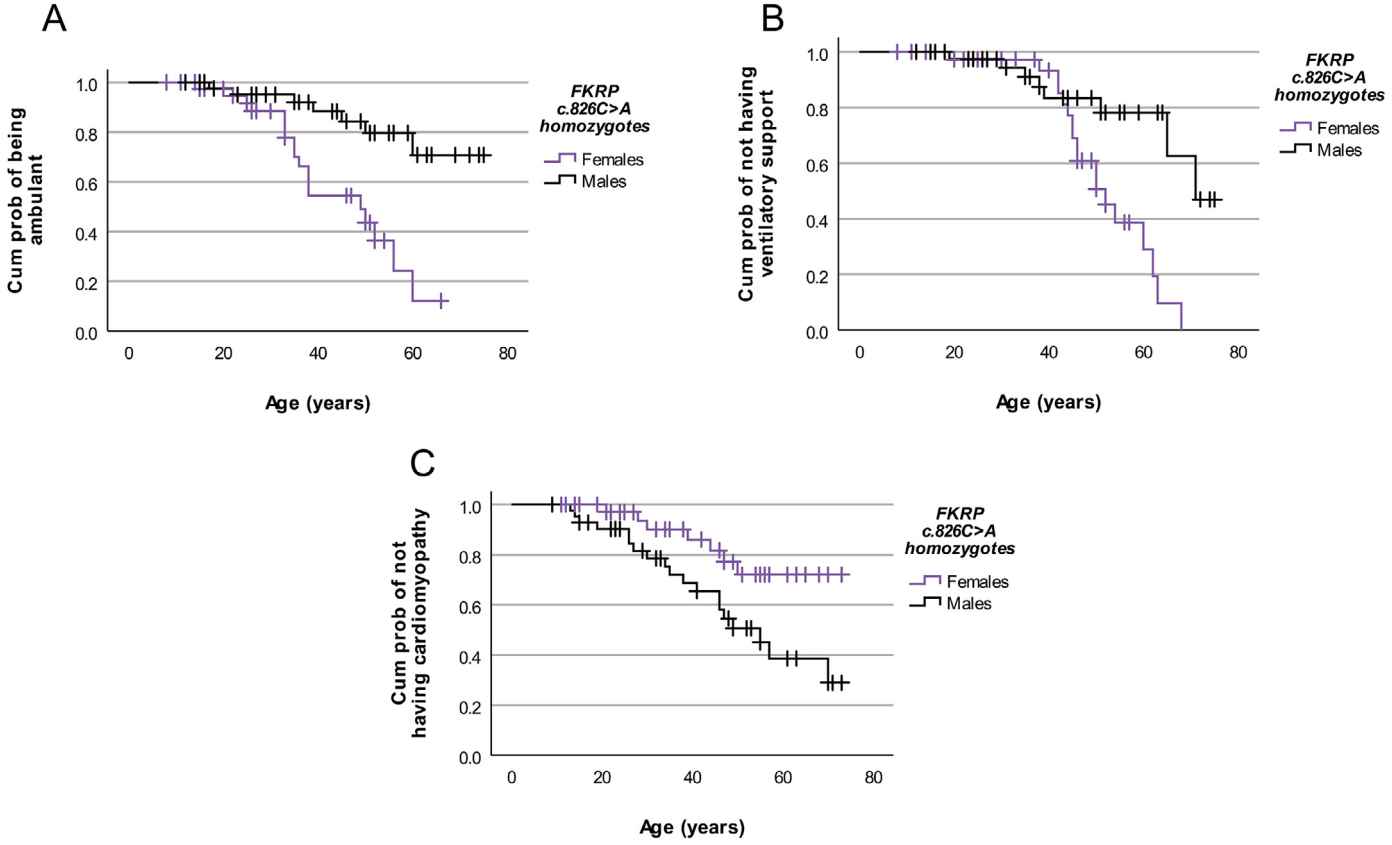

Fig. 3. Kaplan-Meier curves showing cumulative probability with age of (A) being ambulant, (B) not having initiated ventilatory support, and (C) not having cardiomyopathy, respectively, in FKRP c.826C>A homozygous males (n = 45) versus females (n = 43).

(one cardiac transplanted and three with an implanted electronic device) and that three of them had relatively preserved ambulatory function with MCS 2 (cardiac transplanted), 2 and 1 (comorbidity: pulmonary sarcoidosis), respectively. Nevertheless, MCT did not confirm the gender differences.

3.2.4. Natural history of the non-c.826C>A homozygous cohort

The natural history data are summarized in Table 2. The **non**-c.826C>A homozygous group showed earlier symptom onset (median 3 years, p = 0.034) and faster progression than the c.826C>A homozygotes (Fig. 4). Median age at diagnostic work-up was 7 years (IQR 3-20) (p = 0.005). Median time from onset to work-up was 6 years (IQR 2-13) excluding two subjects with incidental finding of elevated CK or transaminases and two who had a previously diagnosed sibling. Further, they demonstrated an increased cumulative probability of wheelchair dependency (HR 9.67 (CI: 4.19, 22.39), p < 0.001) and of requiring ventilatory support (HR 5.39 (CI: 1.84, 15.77), p = 0.002), but no significant increase in respect to developing cardiomyopathy (HR 2.13 (CI: 0.81, 5.64), p = 0.13). The 50 % probability of wheelchair dependency was reached by the age of 20 (CI: 7, 33) and of cardiomyopathy development by 31 years (CI: 24, 38). We could not predict a 50 % cumulative probability of ventilatory support by survival analysis (Fig. A2), but ventilatory support was preceded by

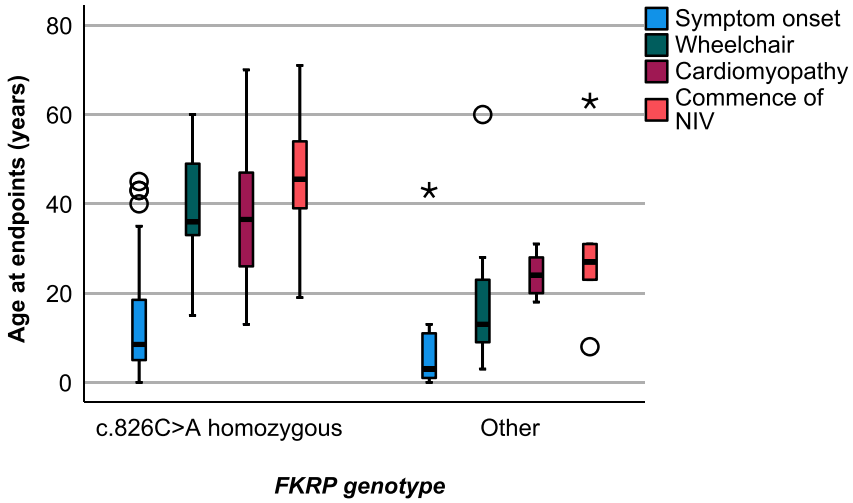

Fig. 4. Box plot showing age at symptom onset, wheelchair dependency, cardiomyopathy and initiation of non-invasive ventilatory support (NIV) in subjects with FKRP c.826C>A homozygous genotype (n = 88) versus other FKRP genotypes (n = 13).

wheelchair dependency in five of five cases with a median interval of 13 years. MCT did not confirm gender difference in age of onset but did so in cumulative probability of wheelchair dependency and ventilatory needs.

3.2.5. Siblings

In order to investigate to which extent clinical heterogeneity was present within families, siblings were compared. Among 12 c.826C>A homozygous sib-ships, median inter-sibling difference in age of onset was 5 years (range 0-10). MCS was similar in most siblings, but highly divergent in two sib-ships (male 36/female 40, male 69/female 71 years old, family number 6 and 7, Table C1). The occurrence of cardiomyopathy appeared randomly since only one sibling was affected in seven of eight cases (Table C1). In the **non**-c.826C>A homozygous cohort, one sib-ship comprising a male and two females with the c.826C>A p.(Leu276Ile) / c.962C>A p.(Ala321Glu) genotype, the male exhibited a more severe course regarding both motor, respiratory and cardiac involvement (Table A, Subject 7, 8 and 9).

4. Discussion

4.1. Prevalence and molecular data

With a minimum prevalence of 2.84/100,000 (adult: 3.06/100,000, paediatric: 1.98/100,000), Norway has the highest reported prevalence of LGMDR9 worldwide. Moreover, we assume that the paediatric prevalence is underestimated due to the delay from symptom onset to diagnostic work-up caused by mild and nonspecific symptoms at onset. The high LGMDR9 prevalence in Norway reflects the high carrier frequency of the *FKRP* c.826C>A allele in the population, which we calculate to 1/101 (total carrier frequency 1/94). This is only slightly higher than the carrier frequency in the Swedish population (1/109) [11], but more than twice the frequency in the non-Finnish, north-western European population (1/218) and 4-5 times the frequency in the Finnish population (1/469) [11]. There are also variations within Norway: the prevalence is relatively high in Northern-Norway and low in the southwest in accordance with previous regional epidemiological studies [16,31]. Additionally, we find a relatively high prevalence in the central region (counties 3 and 4, Fig. 1), and the peak prevalence of 8.32/100,000 in Nordland county in the north (county 2, Fig. 1). Interestingly, a recent study on the genetic distances between Norwegian counties, performed by hierarchical clustering of pair-wise F_{ST} distances, demonstrates that counties 2, 3 and 4 (Fig. 1) group together [32].

As the age and origin of the c.826C>A variant remains unclear, it is difficult to explain the distribution of subjects. A north-south gradient in Europe may be explained by previous waves of European migration northward and eventually into the Scandinavian Peninsula. Small, isolated settlements with little intermixing could then explain why the variant shows a skewed accumulation in Norway. Genetic studies have demonstrated major genetic inflow to Norway from Central and Western European populations, especially the Germanic population [33], but also geographic sub-structuring believed to be partially caused by geographic isolation by mountains and the sea [32,34]. The geographical distribution of subjects within counties appears as scattered clusters (not shown). These clusters likely emerged from migration, followed by historical isolation and subsequent genetic drift. The fact that also the Norwegian c.826C>A homozygotes are homozygous for the *FKRP* c.135C>A allele, previously shown to be in complete linkage disequilibrium with c.826C>A in other populations [9,10], supports the theory that c.826C>A is a founder variant.

4.2. Clinical data

Similar to previous studies, we found that a high proportion of our cohort had calf pseudohypertrophy [9,20,23], tendo-achilles contractures [20,30,35], cardiomyopathy [17,19,36] and need for ventilatory support [19,20,22]. Scapular winging was more frequent than previously reported [20,24,37], although often mild. Overlapping clinical features can be seen with the most common recessive LGMDs including calpainopathy (LGMDR1), dysferlinopathy (LGMDR2), sarcoglycanopathies (LGMDR3-R6) and anoctaminopathy (LGMDR12) [38]. Perhaps the clearest overlap is with the sarcoglycanopathies [39,40], although these tend to show earlier loss of ambulation [39]. In contrast, dilated cardiomyopathy and ventilatory needs are uncommon in subtype R1 [41,42], R2 [43] and R12 [44–46]. Furthermore, anoctaminopathy and dysferlinopathy are associated with a later onset; median 35 years (common type) [44] and 19 years [47], respectively.

Exertional intolerance mimicking metabolic muscle disease was common in our LGMDR9 cohort, and similar to that described in a German LGMDR9 cohort [9]. This is diagnostically relevant, as is the high proportion (18.0 %) in whom the muscle disease was discovered by finding elevated CK or transaminases. That most of these patients also had unrecognized symptoms or signs of muscle disease highlights the need for CK analysis and appropriate clinical examination in patients with elevated transaminases to avoid misinterpretation as liver pathology. This was also addressed in a previous Norwegian study [30]. Pseudometabolic presentation and asymptomatic hyperCKemia are also frequent in anoctaminopathy [46,48]. The significant latency from symptom onset to diagnostic work-up for a muscle disorder, especially in c.826C>A homozygotes (median 10.5 years), may reflect a slow disease progression. Delayed diagnoses was also reported recently in an American LGMDR9 cohort [18]. Similar to previous natural history studies, oropharyngeal symptoms were reported in our cohort: these included two cases with painful tongue cramps [35], one case with dysphagia that resolved after the initiation of ventilatory support [23], one with difficulties masticating food [49], and four with reduced tongue strength [20]. We found that dysphagia and dysarthria tended to develop late in the disease course.

In the patients requiring ventilatory support, OSA was frequently diagnosed (53.1 %), although most often associated with a restrictive ventilatory defect. Whether OSA in these subjects is due to LGMDR9-related upper airway involvment, remains to be elucidated. In muscular dystrophies, the mechanisms of OSA are various, and OSA can be confounded by diaphragmatic events [50]. Furthermore, OSA is multifactorial, commonly related to high weight and increasing age, and prevalent in the general population [51]. As reduced mobility predisposes to weight gain, and several participants did have obesity, weight could be a significant factor. In a cohort of patients with dysferlinopathy, OSA was reported in some patients and considered likely related to age and BMI [43], which highlights the relevance of cofactors in the natural history.

We analysed the disease course in c.826C>A homozygotes separately and found a median age of onset of 8.5 years, which is similar to a US cohort [18]. Further, we found a bimodal distribution with a second peak in the third decade of life raising the possibility of protective genetic or epigenetic factor(s) in the late-onset subpopulation. It should be remembered, however, that age of onset is subjective and defined retrospectively. Defining the age of onset can be difficult in cases of gradual or non-specific symptoms such as poorer physical performance than peers. Also, time of recognition is likely influenced by the level of physical activity. We found that age of onset was not an independent predictor of wheelchair dependency, the need for ventilatory support or the risk of cardiomyopathy.

Both age and symptom duration at onset of wheelchair dependency varied widely. Wheelchair dependency and need for ventilatory support correlated with female gender, and there was an inverse relationship between the ambulatory status and the need for ventilatory support. The gender difference in ventilatory needs was linked to the difference in ambulatory status. The need for ventilatory support usually occurred in the non-ambulatory stage analogous to other muscular dystrophies [52,53]. In sarcoglycanopathy, the need for ventilatory support is related to long disease duration and scoliosis [39]. The link to disease duration was similar in the current study, but the relationship with scoliosis was not assessed. Clinically, scoliosis was infrequent and often mild and since X-ray was usually not performed, it may be underdiagnosed or underestimated. There are few studies of the impact on respiration in patients with LGMDR9, although correlation between disease severity and need for ventilatory support [35], and lack of correlation between muscular involvement and respiratory function have been reported·[19,24]. As in previous studies, our data showed no correlation between cardiomyopathy development and loss of ambulation [19,24,35,36], nor with disease duration or age. We did, however, find a positive correlation with male gender, although significance was not confirmed when we corrected for multiple subgroup analyses. Moreover, the most serious cases of cardiomyopathy in our study occurred in c.826C>A homozygous males. By contrast, in sarcoglycanopathy, the presence of cardiac involvement was found to be related to symptom duration [39].

Clinical gender dissimilarities in LGMDR9 were an unexpected finding. These differences cannot be explained by unequal gender representation, since the numbers of males and females diagnosed with LGMDR9 and their participation rates in this study were similar. A register study did report earlier loss of running ability in females, and the tabulated data show a tendency towards higher wheelchair use and need for ventilatory support in females than males [22]. Interestingly, this is opposite to the tendency reported for other LGMDs: in Anoctamin 5-related LGMD, females appear less frequently affected [44,46] and in both Calpain- and Telethonin-related LGMD, males have more severe muscular impairment [41,54]. Likewise, a morphometric study showed more muscle fiber atrophy in males with Calpain- or Dysferlin-related LGMD, and suggested explanations were endocrinological and differing initial muscle mass [55]. Muscle MRI in LGMDR9 patients has shown some gender-specific patterns of involvement [56] and an MRI study of dysferlinopathy, showed that females had more severe involvement of several muscles of the lower limb [57]. These studies offer potential support for our findings. Gender was also analysed in two previous studies looking at cardiac involvement in LGMDR9, albeit in genotypically heterogenous cohorts. In one study an increased tendency for cardiomyopathy was found in males [19], while in another study no association with gender was identified [17].

Gender-specific differences may also have explanations unrelated to the underlying muscle disease. Different self-reporting behavior, recognition and comorbidity can play a role. For example, levels of ambulation were mostly based on self-reported data. Recognition of ventilatory needs depends on the symptoms the patients report and their motivation to undergo a sleep study. Additionally, BMI could be a significant factor in the need for ventilatory support, and our data did indicate that obesity was more frequent in female participants. The increased risk of cardiomyopathy in males may also be related to comorbidity and excluding other etiologies such as cardiovascular disease (CVD), alcohol overconsumption or viral myocarditis, is a challenge. Males, in general, have higher incidence of CVD, partly explained by the sex hormones [58], but CVD may be under-recognized in females [59]. Accordingly, our comorbidity data showed a tendency of more CVD in males. Lastly, true LGMDR9-related gender differences can still be influenced by sex hormones and life style. Although it has been suggested that estrogen has a protective role of the skeletal muscle membrane [60], there may be other relevant effects of sex hormones in LGMDR9. The fact that the skeletal and cardiac muscle involvement showed opposite relation to gender may indicate that these tissues are influenced by different factors. One could also hypothesize a cause-effect link where preserved ambulation leads to more physical exposure, which in turn puts strain on the cardiac muscle. Nutrition including deficiencies, metabolic health and exposure to toxins may also play a role in the disease progression.

In the genotype-phenotype analyses our data were skewed with the **non-**c.826C>A homozygous subjects being relatively underrepresented and genetically heterogeneous. As expected from previous studies, **non**-c.826C>A homozygous subjects demonstrated a more severe disease course, although mild

phenotypes or late onset of disease did also occur. While the numbers in our study were small, the risk of developing cardiomyopathy was not significantly increased in the **non**-c.826C>A homozygous cohort. Previous studies have suggested both a higher risk [17,19], and no difference in risks for developing cardiomyopathy [36].

We compared the 13 sib-ships among participants to address the question of environmental influence and found variability in age of onset (up to ten years), progression and cardiac involvement. Inter-sibling variability was also documented previously [10,23,24] and suggest that other genetic factors and/or environmental factors may play a role in disease development.

4.3. Strengths and limitations

With 101 participants in the observational study, our sample size is the largest outside the global register. A participation rate of 66 % is high but may still limit the generalization of the results. Our cohort had equal gender representation, paediadric and adult subjects, and data from all the Norwegian counties. The substantial number of c.826C>A homozygotes enabled us to perform a genotype-specific natural history analysis. Patient notes provided long-term data and, combined with the questionnaire, strengthened the data completeness and quality. Neuromuscular examination of 42.6 % of the participants compensated in part for incomplete data in patient notes.

The validity of data from patient notes is limited by the non-standardized follow-up and reporting, and consequently possible inaccuracies and underestimation of outcomes. Furthermore, when concerning cardiomyopathy, normal limit of LVEF was set to 50 % to agree with the echocardiography reports. Both 50 and 55 % are used in other studies. Considering the trend towards increased use of more sensitive technology, such as MRI, and a more liberal LVEF-threshold for cardioprotective treatment initiation, our results are conservative. Conversely, some cases with cardiomyopathy considered to be LGMDR9-related may have another etiology and thereby overestimate the risk. The lack of specific markers of LGMDR9-related cardiomyopathy is thus a limitation. Data concerning ambulation relied mainly on self-reports, which opens to misinterpretation and bias. In addition, we acknowledge that implementation of a validated scale for self-reported ambulation would be preferable, and objective measures optimal. The gender differences need validation through clinical testing, and the cardiorespiratory involvement needs to be assessed with a standardized protocol to obtain more accurate and comparable data. This is a work in progress, however only on the subset of participants who also consented to clinical participation. Analyses of the **non**-c.826C>A homozygous group were limited by power due to small sample size and genotype heterogeneity, and included one subject with a likely pathogenic variant. Elimination of this participant did not change the conclusions of the subgroup comparisons.

5. Conclusions

The Norwegian LGMDR9 prevalence remains the highest reported worldwide and is strongly linked to the high prevalence of the c.826C>A founder variant. Our study extends understanding of the clinical features and natural history of LGMDR9, particularly the phenotypes associated with c.826C>A homozygosity. Our data showed that initial symptoms commonly occur in the first decade of life, and there is a significant latency from onset to diagnostic work-up. This may indicate the need for increased awareness of muscle disorders in children. Both lower limb weakness and exertional myalgia were common early symptoms. Further, our results indicate that respiratory follow-up is increasingly important particularly as patients become wheelchair dependent, but that sleep-disordered breathing occurs to an uncertain extent also in ambulatory patients. Our data also support the importance of regularly cardiac assessment. The study provides insight that should be relevant to future and ongoing LGMDR9 clinical trials. The bimodal distribution of onset, the lack of correlation between skeletal and cardiac muscle involvement, and the gender correlations, are relevant findings in the search for disease modifying factors.

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Declaration of Competing Interest

None

Acknowledgment

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Appendices

Fig. A1

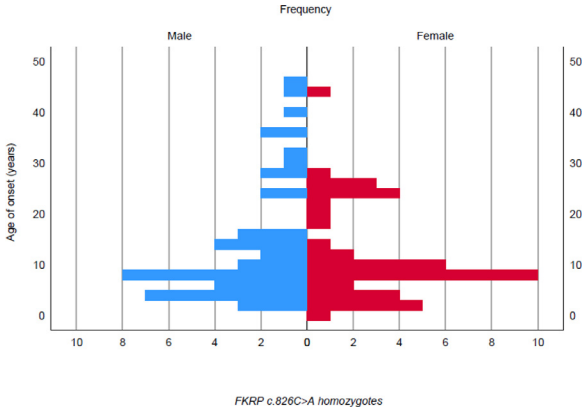

Fig. A1. Histogram demonstrating distribution of age at onset in the FKRP c.826C>A homozygous participants divided into genders. (For interpretation of colour please refer to online publication.)

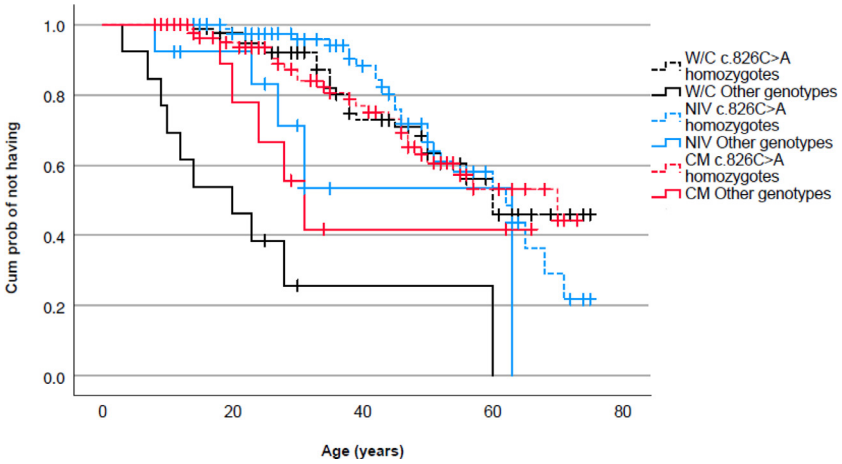

Fig. A2. Kaplan-Meier curves showing cumulative probability with age of not being wheelchair dependent, initiated non-invasive ventilatory support or developed cardiomyopathy, respectively, in FKRP c.826C>A homozygotes versus the cohort of other FKRP genotypes. (For interpretation of colour please refer to online publication.)

Table A1
Genotype-phenotype table of non-*FKRP* c.826C>A homozygous participants

No.	*FKRP* genotype [references of earlier identified variants]	Gender	Age	MCS	Age at onset (years)	Spine	Contr	Age at W/C (years)	Age at CM (years)	Age at NIV (years)	PEG
	Non c.826C>A:										
1.	c.160C>T p.(Arg54Trp) homozygous [1]	F	11	9	0	+	-	9	-	-	-
	c.826C>A compound with:										
2.	c.166T>A p.(Phe56Ile)	M	24	4	13	-	-	-	20	-	-
3.	c.141_151 del 11 p.(Arg48Profs*9)	F	11	8	0	+	-	10	-	-	-
4.	c.328C>T p.(Arg110Trp) [2]	M	29	0	7	-	-	-	-	-	-
5.	c.899T>C p.(Val300Ala) [3–7]	M	65	9	43	+	-	60	-	63	-
6.		F	24	0	11	-	-	-	‡	-	-
7.†	c.962C>A p.(Ala321Glu) [7,8]	F	29	9	12	+	-	23	24	-	-
8.†		F	34	9	8	+	-	20	-	-	-
9.†		M	36	9	2	+	+	7	18	23	+
10.		F	34	9	3	+	+	14	31	27	+
11.		M	62	9	1	+	+	12	-	31	+
12.	c.1323T>G p.(Phe441Leu) [5]	F	9	6	1	+	-	3	-	8§	-
13.		F	30	8	1	-	-	28	28	-	-

MCS = ambulation score from 0 (normal) to 9 (= lost ambulation) (Details; Sect. 3.4), spine = spine deformities, contr = contractures in upper and lower limbs, W/C = wheelchair dependency, CM = cardiomyopathy, NIV = initiation of non-invasive ventilatory support, PEG = Percutaneous Endoscopic Gastrostomy, F = female, M = male

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†Siblings, ‡Lacking cardiac follow-up, § Indication: fatigue with slight sleep apnea

Table B1
Motor composite score (MCS) (n = 88)

	Unadjusted β (CI)	Unadjusted p-value	Adjusted β (CI)	β relative change (%)	Adjusted p-value
AGE (years)	0.09 (0.06, 0.12)	< 0.001*	0.03 (-0.02, 0.08)	- 67	0.21
YEARS SYMPTOMATIC	0.10 (0.08, 0.13)	< 0.001*	0.08 (0.03, 0.08)	- 23	0.001*
GENDER (M)	-1.52 (-2.82, - 0.23)	0.022*	-1.69 (-2.70, -0.69)	- 11	0.001*

R^2 = 0.67, Z-residuals < ± 3, Cooks distances << 1.0.
MCS: ambulation score from 0 (normal) to 9 (=lost ambulation) (Details: Sect. 3.4), · p < 0.05, β = regression coefficient, CI = 95 % confidence interval, M = male gender. Unadjusted effects and p-values are calculated by simple regression analyses. Adjusted values mean that it is controlled for the other variables in the table.

Table B2
Wheelchair dependency (n = 25/88 (28 %))

	Unadjusted OR (CI)	Unadjusted p value	Adjusted OR (CI)	OR relative change (%)	Adjusted p-value
AGE (years)	1.05 (1.02, 1.09)	< 0.001*	1.02 (0.96, 1.08)	- 3.1	0.55
YEARS SYMPTOMATIC	1.06 (1.03, 1.09)	< 0.001*	1.05 (0.99, 1.11)	- 0.66	0.060
GENDER (M/F)	0.26 (0.09, 0.70)	0.006*	0.17 (0.05, 0.55)	- 35	0.0015*

Nagelkerke R^2 = 0.38. Hosmer and Lemeshow Test: p = 0.34. Cookś distances << 1.0. Three Z-residuals > ± 3 (3.6 - 4.5) - removal showed only small differences in effect sizes, although adjusted p-value of "Years symptomatic" dropped below 0.05.
*p < 0.05, OR = Odds ratio, CI = 95 % confidence interval, M = males, F = females. Unadjusted effects and p-values are calculated by simple regression analyses. Adjusted values mean that it is controlled for the other variables in the table.

Table B3
Initiated ventilatory support (n = 27/88 = 31 %)

	Unadjusted OR (CI)	Unadjusted p-value	Adjusted OR (CI)	OR relative change (%)	Adjusted p-value
AGE (years)	1.08 (1.04, 1.12)	< 0.001*	1.11 (1.04, 1,20)	+ 3.2	0.0011*
YEARS SYMPTOMATIC	1.06 (1.02, 1.09)	< 0.001*	(0.90, 1.01)	- 9.6	0.11
GENDER (M/F)	0.31 (0.12, 0.82)	0.015*	0.41 (0.10, 1.64)	+ 33	0.21
MCS	1.68 (1.36, 2.08)	< 0.001*	1.63 (1.21, 2.19)	- 3.3	0.0003*

Nagelkerke R^2 = 0.58. Hosmer & Lemeshow Test: p = 0.52. Z-residuals < ± 3. Cookś distances << 1.0.
*p < 0.05, OR = Odds ratio, CI = 95 % confidence interval, M = males, F = females, MCS: ambulation score from 0 (normal) to 9 (= lost ambulation) (Details: Sect. 3.4). Unadjusted effects and p-values are calculated by simple regression analyses. Adjusted values mean that it is controlled for the other variables in the table.

Table B4
Cardiomyopathy (n = 26/84 (31 %))

	Unadjusted OR (CI)	Unadjusted p-value	Adjusted OR (CI)	OR relative change (%)	Adjusted p-value
AGE (years)	1.01 (0.98, 1.03)	0.58	0.97 (0.92, 1.02)	-4.0	0.21
YEARS SYMPTOMATIC	1.02 (0.99, 1.04)	0.24	1.05 (0.99, 1.11)	+3.2	0.092
GENDER (M/F)	3.85 (1.40, 10.58)	0.006*	3.71 (1.25, 11.00)	-3.5	0.014*
MCS Q		0.69			0.75
Q2	0.96 (0.24, 3.93)	0.96	0.65 (0.14, 3.10)	-32	0.59
Q3	1.66 (0.43, 6.38)	0.46	1.25 (0.26, 6.00)	-25	0.78
Q4	0.80 (0.20, 3.22)	0.75	0.64 (0.10, 3.99)	-20	0.64

Nagelkerke R^2 = 0.17. Hosmer & Lemeshow Test: p = 0.99. One Z-residual > ± 3 (3.1). Cookś distances << 1.0.
*p < 0.05, OR = Odds ratio, CI = 95 % confidence interval, M = males, F = females, MCS: ambulation score from 0 (normal) to 9 (= lost ambulation) (Details: Sect. 3.4), MCS Q = MCS quartile, Q2 = MCS 2-3, Q3 = MCS 4-7, Q4 = MCS 8-9. Unadjusted effects and p-values are calculated by simple regression analyses. Adjusted values mean that it is controlled for the other variables in the table.

Table C1
Natural history of sib-ships of patients homozygous for FKRP c. 826C>A

Family No.	Gender	Age (years)	MCS	Age of onset (years)	Age at W/C (years)	Age at CM (years)	Age at NIV (years)
1.	F	46	4	10	-	44	-
1.	F	50	3	20	-	-	-
2.	F	33	1	28	-	-	-
2.	M	29	0	24	-	27	-
3.	M	35	3	7	-	-	-
3.	M	31	1	13	-	-	-
4.	F	25	3	6	-	-	-
4.	M	23	4	13	-	-	-
5.	M	46	3	7	-	46	-
5.	M	49	4	13	-	-	-
6.	M	69	2	15	-	49 (Transplant at 57)	65
6.	F	71	9	7	50	-	62
7.	M	36	1	32	-	-	-
7.	F	40	9	25	38	-	-
8.	M	16	2	10	-	-	-
8.	F	20	2	11	-	-	-
9.	M	55	2	7	-	-	-
9.	F	51	5	4	-	-	44
10.	M	35	6	1	-	34	-
10.	F	30	7	1	-	28	-
11.	M	18	1	12	-	14 (or myocarditis?)	-
11.	F	12	0	9	-	-	-
11.	F	11	1	9	-	-	-
12.	M	56	9	3	45	-	30
12.	M	53	8	3	35	38 (ICD at 38)	38

MCS = ambulation score from 0 (normal) to 9 (= lost ambulation) (Details; Sect. 3.4), W/C = wheelchair dependency, CM = cardiomyopathy, NIV = initiation of non-invasive ventilatory support, F = females, M = males, ICD = implantable cardioverter-defibrillator
-Signifies that the outcome was not reached

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