UiT The Arctic University of Norway

Faculty of Health Sciences

FKRP-related limb-girdle muscular dystrophy R9 in Norway – Studies of epidemiology, natural history, and relationships with health-related quality of life and sleep

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List of abbreviations

AASM	American Academy of Sleep Medicine
AHI	Apnea-hypopnea index
BIS	Bergen Insomnia Scale
CBT, CBT-I	Cognitive behavioral therapy, CBT for insomnia
CO2	Carbon dioxide
DSM	Diagnostic and Statistical Manual of Mental Disorders
EDS	Excessive daytime sleepiness
ESS	Epworth Sleepiness Scale
FKRP, *FKRP*	Fukutin-related protein, FKRP gene
FSS	Fatigue Severity Scale
FVC	Forced vital capacity
HRQoL	Health-related quality of life
ICSD, ICSD-3	International Classification of Sleep Disorders, ICSD-third edition
INQoL	Individualized Neuromuscular Quality of Life
LGMD, LGMDR9	Limb-girdle muscular dystrophy, FKRP-related LGMD R9
MIP, MIP%	Maximal inspiratory pressure, MIP percent predicted
NMD	Neuromuscular disorders
OSA	Obstructive sleep apnea
PAP	Positive Airway Pressure
PSG	Polysomnography
PSQI	Pittsburg Sleep Quality Index
PtcCO2	Transcutaneous CO2 tension
QoL	Quality of life
REM	Rapid eye movement
SA	Sleep apnea
SDB	Sleep-disordered breathing
SF-36	36-Item Short Form Health Survey
SNP	Single-nucleotide polymorphism

List of papers

Paper I

Jensen SM, Müller KI, Mellgren SI, Bindoff LA, Rasmussen M, Ørstavik K, Jonsrud C, Tveten K, Nilssen Ø, Van Ghelue M, Arntzen KA (2023) Epidemiology and natural history in 101 subjects with FKRP-related limb-girdle muscular dystrophy R9. The Norwegian LGMDR9 cohort study (2020). Neuromuscul Disord. 33(2):119-132. doi: 10.1016/j.nmd.2022.11.005.

Paper II

Jensen SM, Friborg O, Mellgren SI, Müller KI, Bergvik S, Arntzen KA. Health-Related Quality of Life in FKRP-Related Limb-Girdle Muscular Dystrophy R9 (2024) J Neuromuscul Dis. 11(1):59-74. doi: 10.3233/JND-221629.

Paper III

Jensen S, Abeler K, Friborg O, Rosner A, Olsborg C, Mellgren SI, Müller KI, Rosenberger AD, Vold ML, Arntzen KA (2024) Insomnia and sleep-disordered breathing in FKRP-related limb-girdle muscular dystrophy R9. The Norwegian LGMDR9 cohort study (2020). J Neurol. 271(1):274-288. doi: 10.1007/s00415-023-11978-7.

Abstract

Background/aims: Limb-girdle muscular dystrophy R9 (LGMDR9) is a rare autosomal recessive muscle disease caused by mutations in the fukutin-related protein gene, *FKRP*. The prevalence is higher in northern European populations, where it is closely associated with the *FKRP* c.826CA allele. This work aims to study the epidemiology of LGMDR9 in Norway, natural history, and relationships with health-related quality of life (HRQoL) and sleep in the Norwegian LGMDR9 population. ***Methods:*** Subjects with genetically confirmed LGMDR9 were identified through Norwegian hospital departments and invited to "The Norwegian LGMDR9 cohort study". A questionnaire and patient notes were collected from consenting persons. Adults were also invited to the University hospital of North Norway for a battery of tests, and to postal surveys on HRQoL, fatigue, and sleep. The HRQoLs were administered three times during a 14-month period. ***Results:*** A total of 153 subjects were identified, of whom 88% were c.826C>A homozygotes. The county-level prevalence ranged 0.63-8.32/100,000 and was highest in Northern and Central Norway and lowest in south-west Norway. Among c.826C>A homozygotes, females showed showed an increased cumulative probability of wheelchair dependency and need for Positive Airway Pressure (PAP) therapy, whereas males were more predisposed to cardiomyopathy. Females reported higher LGMDR9-related burden than males. Both physical, social, and mental HRQoL were impaired. During the 14-month period, perceived muscle weakness and LGMR9 burden worsened in c.826C>A homozygotes. Burden was related to perceived muscle weakness and fatigue. Fatigue was prevalent (40%), associated with insomnia, and correlated with level of mental distress, myalgia, and inspiratory weakness. Insomnia was prevalent both among subjects with and without PAP therapy and was negatively correlated with mental HRQoL. Among subjects without PAP therapy, undiagnosed sleep apnea was frequent, and the apnea-hypopnea index correlated with advancing age and cardiac failure. ***Conclusion:*** Northern and Central Norway have the highest recorded prevalence of LGMDR9 worldwide. The study results suggest sex differences in natural history that future studies should pay attention to. While disease-modifying treatment is still not available, the findings suggest a need for improvements in social and mental areas of HRQoL, attention to gender-specific care needs, and improved identification and treatment of fatigue. Furthermore, the findings suggest that insomnia, myalgia, mental distress, and respiratory muscle weakness are potential treatment

targets for fatigue, and hence should be addressed in fatigued patients. Insomnia and sleep apnea appear to be underrecognized in patients with LGMDR9.

1 Introduction

1.1 FKRP-related limb-girdle muscular dystrophy R9 - Classification and pathogenesis

Limb-girdle muscular dystrophies (LGMD) constitute genetically and phenotypically a heterogeneous group of muscle wasting disorders that primarily affects the muscles of the pelvic and shoulder girdles and are inherited in an autosomal manner. The term was introduced by Walton and Nattrass in the 1950s [1] - before the era of molecular genetics, which began in the late 80s, when the gene and protein of Duchenne muscular dystrophy were first discovered [2]. In the early 90s, the first LGMD genes were identified, and in 1995 the first LGMD classification became established [3]. The LGMD subtype was defined by mode of inheritance, denoted by 1 for autosomal dominant and 2 for autosomal recessive, and the causative gene or genetic locus denoted by letter according to the order of discovery. In 2018, the alphabet was becoming too short for the increasing number of subtypes, and consequently the LGMD nomenclature had to be revised. Inheritance then became denoted by letter instead of number (1 -> D for “dominant” and 2 -> R for “recessive”), the gene by number instead of letter, and the name of the related protein was added [4]. Additionally, new LGMD criteria were proposed in addition to autosomal inheritance and predominantly proximal weakness [4]: The condition should have been described in at least two unrelated families. Additionally, dystrophic changes on muscle histology, increased creatine kinase activity, and degenerative findings on muscle imaging should have been demonstrated. Lastly, achievement of independent walking was required to separate LGMD from congenital muscular dystrophy.

According to the nomenclature described above, FKRP-related LGMD R9 (LGMDR9, formerly designated LGMD2I) is an autosomal recessive LGMD related to the fukutin-related protein (FKRP) gene (*FKRP*). *FKRP* was first discovered by Brockington and colleagues in 2001 in families with congenital muscular dystrophophy [5]. FKRP is a Golgi-resident glycosyl (ribitol 5-phosphate) transferase involved in the post-translational O-glycosylation of α-dystroglycan and the N-glycosylation of fibronectin, which are extracellular membrane

proteins essential for anchoring the muscle membrane to laminin and collagen, respectively, in the extracellular matrix [6-9].

In Online Mendelian Inheritance in Man, LGMDR9 is classified as muscular dystrophy-dystroglycanopathy type C5 (MDDGC5). At least 18 genes are associated with dystroglycanopathy [10]. Several of these express a clinical continuum including LGMD, congenital muscular dystrophy, muscle-eye-brain disease, and Walker-Warburg syndrome [11]. This also applies to *FKRP* [5, 12, 13]. In LGMDR9, cognitive impairment is only sporadically reported, and occasional cases of cognitive impairement may potentially have other causes. Cerebral MRI performed in small samples with LGMDR9 has not demonstrated structural abnormalities [12, 14-17]. However, one study suggested a mild visuospatial dysfunction in LGMDR9 [17], and another one dysfunction of the cone bipolar cells in the retina [18]. The pathogenesis of the dystroglycanopathies in the central nervous system is largely unknown [11]. In Fukuyama congenital muscular dystrophy, findings suggest that deficient fukutin causes dystroglycan-related membrane abnormalities in the astrocyte endfeet, interfering with neuronal migration, and additionally, by presynaptic roles in the neurons, it may cause epilepsy and formation of neurofibrillary tangles [19].

1.2 Prevalence

The reported prevalence of LGMD ranges from 0.8 to 7 per 100,000 people [20, 21]. LGMD is recognized as the fourth most prevalent type of muscular dystrophy after the dystrophinopathies, myotonic dystrophy, and facioscapulohumeral muscular dystrophy. There are regional variations at the subtype level. While calpain-related LGMD R1 is the most reported LGMD globally, dysferlin-related LGMD R2 is recognized to predominate in US, Japan, and Mexico, sarcoglycan-related LGMD R5-8 in India, anoctamin 5-related LGMD R12 in Finland, and LGMDR9 in Scandinavia [21]. LGMDR9 is relatively prevalent in North-European populations. This is closely related to the spread of the disease-causing variant *FKRP* c.826C>A (p.L276I), since it is almost invariably present in one or both alleles in LGMDR9 populations of North-European descent [12, 16, 22-24], whereas a different genetic spectrum was found e.g., in Asia [25]. Additionally, genomic database indicates that the *FKRP* c.826C>A allele is rarer in other populations and not detected in Asia [26]. Genetic studies of small samples in Germany [16] and the Hutterites, who migrated from North-Europe to Canada in the 1870s [22], showed that this variant was 100% associated and in

linkage disequilibrium with an intragenetic single-nucleotide polymorphism (SNP), *FKRP* c.135C>T. This suggests that *FKRP* c.826C>A is a founder variant (i.e., deriving from a common ancestor) rather than a hotspot mutation (i.e., resulting from recurrent events). Other founder variants have also been identified in LGMDR9 [27, 28].

The highest recorded prevalence of LGMDR9 worldwide has been in Norway in 2009 (1.85/100,000) [24] and in Northern Norway, comprising ~10% of the Norwegian population, in 2020 (5.88/100,000) [29]. In Hordaland, a previous county in south-west Norway (currently a part of Vestland county), also comprising ~10 % of the Norwegian population, the prevalence in 2013 was relatively low (0.8/100,000) [30]. Genetic testing for LGMDR9 became first available in Norway in 2006.

1.3 Natural history

Besides limb-girdle distribution of muscle weakness, the LGMDR9 phenotype has been characterized by pseudohypertrophic calves, tendo-achilles contractures, macroglossia, hyperCKemia, dilated cardiomyopathy, respiratory involvement, and sometimes scapular winging, scoliosis, and multiple contractures [15, 16, 23, 31]. Some researchers have reported prominent myalgia [16, 32].

Most natural history studies have been conducted in Northern Europe and US where samples comprise mostly *FKRP* c.826C>A homozygotes and *FKRP* c.826C>A compound heterozygotes. Several of these studies have reported that the *FKRP* c.826C>A homozygous genotype is associated with a relatively later onset and milder progression compared to the *FKRP* c.826C>A compound heterozygous genotype, but displays a vast variation in age of onset and severity [14, 15, 23, 24]. Genotype-phenotype variability has also been noted in other LGMDR9 genotypes [33], between siblings with LGMDR9 [15, 22, 34, 35], and in other LGMD subtypes, e.g., α-sarcoglycan-related LGMD [36], suggesting that other genes or epigenetic factors are involved. Furthermore, a study of *FKRP* c.826C>A homozygotes showed that clinical severity neither correlated with pathological findings in muscle biopsies, which supports that the pathogenesis is complex [37].

Data in a global registry with the largest LGMDR9 sample in history (> 300 registrants) show an average age of onset of 19 years in *FKRP* c.826C>A homozygotes compared to 7 years in *FKRP* c.826C>A compound heterozygotes [38]. In a LGMDR9 cohort in US, onset was

reported to be median 9 years in *FKRP* c.826C>A homozygotes and median 2 years in people with other genotypes [39]. In a previous Norwegian study, age of onset among *FKRP* c.826C>A homozygotes ranged 0 to 50 years [24]. Furthermore, global registry data indicate that age of loss of ambulation is highly variable and that *FKRP* c.826C>A homozygotes tend to retain ambulation longer and begin noninvasive ventilation later [38]. The respiratory involvement in LGMDR9 appears relatively uncharacterized. Potential mechanisms in neuromuscular disorders (NMD) include respiratory muscle weakness, chest wall restrictiveness due to scoliosis and stiffening of the ribcage, and upper airway involvement [40]. Some studies on LGMDR9 have reported that noninvasive ventilation is related to disease severity [14, 24, 33], whereas other researchers did not find a clear relationship between motor and respiratory involvement [31, 35].

Concerning LGMDR9-related cardiomyopathy, multiple studies have been conducted. In a Danish LGMDR9 cohort (genotypes unspecified), a significant annual drop in the left ventricular ejection fraction was detected [41]. Reported estimates of 50% probability of cardiomyopathy were by age ~ 50 years in *FKRP* c.826C>A homozygotes compared to ~ 20 years in subjects with other genotypes [31, 42]. Another study reported no difference in cardiac involvement between *FKRP* c.826C>A homozygotes and *FKRP* c.826C>A compound heterozygotes [43]. Individuals with need for an implantable cardioverter-defibrillator [41] or a cardiac transplant [31, 44, 45] have been reported, including three *FKRP* c.826C>A homozygotes with mild [44] or none [45] skeletal muscle involvement. Correspondingly, several studies have reported that cardiac and motor function are variably affected [14, 31, 35, 42, 43]. Similar has been observed in sarcoglycanopathy, and it has been proposed an additional cardiovascular mechanism, since sarcoglycan and dystroglycan are present in both cardiac and smooth muscle cells [46]. Nevertheless, in dystroglycanopathy, it was concluded that the loss of dystroglycan function in cardiomyocytes was capable of inducing a progressive dilated cardiomyopathy, whereas the loss of dystroglycan function in smooth muscle was not [46]. Cardiomyopathy in LGMDR9 has also been found to be unrelated to respiratory function and sex [42].

Little has been reported about sex differences in the natural history of LGMDR9, but global registry data suggest that females lose their running ability earlier than males [38]. Additionally, an MRI study of lower limb showed certain sex-specific patterns [47]. Comparatively, an MRI study on dysferlinopathy showed that females had more severe

involvement in lower limb [48], whereas in anoctamin 5- [49, 50], calpain-, and telethonin-related LGMD [51, 52] and in FSHD [53] males seem relatively more severely affected. The findings are thus conflicting and so far not understood. In FSHD, preliminary data suggest an inhibitory role of estrogens on the toxic effects of DUX4 expression in the muscle [53].

To prepare for clinical trials, longitudinal outcome studies have been conducted in LGMDR9. In a European cohort of ambulant *FKRP* c.826C>A homozygous adults, annual worsening on quantitative MRI and forced vital capacity (FVC) was demonstrated, but not on motor function [54]. In a US cohort [55], annual deterioration of both FVC and motor function was demonstrated in adult *FKRP* c.826C>A homozygotes and in adults with other genotypes. The pediatric US cohort demonstrated more annual worsening than the adult cohort on certain motor outcomes, but less on others. The latter was assumed to be due to normal growth.

1.4 Therapy and trials

While some breakthroughs have occurred for Spinal muscular atrophy (SMA) and Duchenne muscular dystrophy when it comes to causal therapy (antisense oligonucleotide therapy, and for SMA also Adeno-Associated Virus (AAV)-mediated gene replacement therapy), there is currently no disease-modifying therapy available for LGMD. However, clinical trials for LGMD are emerging. In LGMDR9, there is an ongoing Phase 1/2 trial on AAV-mediated gene replacement therapy in Europe (ClinicalTrials.gov Identifier: NCT05224505), and another one in US (NCT05230459). Another genetic approach is autologous cell therapy with CRISPR/Cas9-mediated gene editing [56], and a Phase 1/2 trial on LGMD is scheduled for recruitment 2024 (NCT05588401). The causal therapies have so far only showed modifying effects.

Non-genetic strategies are also being investigated in LGMDR9, including a Phase 3 trial on ribitol supplementation (NCT05775848). The idea is to create an excess of the enzyme substrate to partially compensate for the defective glycosylation of α-dystroglycan [57, 58]. Moving forward, other potential targets for modulating therapy are the fibronectin glycosylation [9], the catabolic pathways [59, 60], and the immunological responses [61]. A Phase 1b/2 trial on myostatin inhibition to stimulate muscle growth has been conducted in LGMDR9 but not found efficacious (NCT02841267). Furthermore, a trial on steroids, which hava a non-specific, complex approach [62], was initiated in LGMDR9 but terminated due to

COVID-19 (NCT03783923). To date, the documentation on steroid responsiveness in LGMDR9 is anecdotical [63-65]. In dysferlinopathy, deflazacort showed even paradoxical effects [66].

Supportive management in LGMD is aimed at preventing and managing physical and cardiorespiratory complications and optimizing daily functioning and health-related quality of life (HRQoL) [67]. Short-term data suggest that moderate-intensity aerobic and strength training is beneficial in LGMD [68], but attention to warning signs such as myoglobinuria or persisting or excessive soreness is recommended [67]. Cardiac assessments with electrocardiogram and echocardiography are advised at diagnosis and every other year by routine, considering the unknown risk factors of cardiac involvement and the importance of timely intervention [69]. International guidelines for respiratory care in LGMD have not yet been established.

1.5 Health-related quality of life

1.5.1 Definition

Health, quality of life (QoL), and HRQoL are frequently confused concepts in the literature [70]. All three are universal constructs with abstract content. By agreed definitions of abstract phenomena one can use indicators to indirectly measure and evaluate such phenomena. Measurement and evaluation of health, QoL, or HRQoL can be used to detect inequalities, needs, or meaningful effects of structural or treatment interventions. The World Health Organization (WHO), at its establishment in 1948, defined health as «a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity» [71]. This definition has been highly influential and contributed to the shift from a naturalistic biomedical approach to a holistic biopsychosocial approach in health care and medical science [72]. In 1995, WHO defined QoL as “an individual´s perception of their position in life in the context of the culture and value systems in which they live and in relation to their goals, expectations, standards and concerns» [73]. From WHO´s definition of health and QoL, respectively, an existing definition of HRQoL is functioning and well-being in physical, psychological, and social aspects of health [70]. Nevertheless, no universal definition exists, and it has been argued that most HRQoL instruments measure the self-perceived health status rather than HRQoL by not including patient valuation [70]. Whereas the idea of QoL or good

life has been traced back to Aristotle [74], the concept of HRQoL first evolved in the 1970s to assist in priorities in public health services as new technology brought new options and demands that raised economical and ethical concerns [75]. Nevertheless, "QoL" was the first term used [75].

1.5.2 Instruments

HRQoL measures can be divided into generic and disease-specific measures, and generic further into profile and preference-based measures. Generic measures can be used across different disease populations and in general populations. Preference-based measures are constructed for estimation of quality-adjusted life years and can thus be used for utility analyses. Profile measures are designed to yield score on various aspects of HRQoL. Disease-specific HRQoLs assess the perceived burden of a specific condition, e.g., by asking "Does your muscle disease make you feel depressed?". The Individualized Neuromuscular Quality of Life questionnaire (INQoL), which was applied in the present study, was the first disease-specific HRQoL measure developed for NMD [76] and is one of the most widely used instruments for adults with NMD.

HRQoL measures are commonly multi-item scales including both physical, mental, and social dimensions of health, in accordance with WHO´s definition of health [77]. Compared to scales with one global question, multi-item, multi-dimensional scales provide more information and are generally more reliable and valid, but on the negative side more burdensome to complete and more complicated to analyze [77]. While the earliest HRQoL were extensive to optimize precision and sensitivity, more feasible versions have been developed. As an example, in 1992, the 36-item Short Form Health Survey (SF-36) was constructed by the US non-profit research organization, RAND Corporation, as a more efficient scale in the Medical Outcomes Study compared to the previous one used in their health insurance experiment (1971-86), and since then, even abbreviated versions of the SF-36 have been constructed [77-79].

1.5.3 HRQoL in neuromuscular disorders

A systematic review conducted in 2010 identified 26 studies on HRQoL in muscle disease [80]. The earliest publication included was a Swedish study in 1994 that assessed relationships between HRQoL and cardiorespiratory involvement in muscular dystrophies

[81]. The review found that both physical, mental, and social aspects of HRQoL were impaired in people with muscle disease and that common negative predictors of HRQoL were disease severity, pain, fatigue, and negative emotions [80]. HRQoL has been assessed in two samples with LGMD specifically: one in Serbia [82] and another with recruitment from international registries [83]. Both studies also included LGMD that was not genetically confirmed and LGMD subtypes that became omitted from LGMD after the recent reclassification, such as Pompe disease and Rippling disease [4]. In Norway, little is known about HRQoL in adults with muscle disease, apart from Myotonic dystrophy type 1 [84-86]. Since the burden of disease is influenced by several external factors, such as culture, technology available, treatment options, and welfare system, it can change with time and place.

1.6 Fatigue

1.6.1 Definition

From a physiological perspective, fatigue can be defined as an organic functional impairment due to depletion of required energy substrates or signaling molecules [87], as in metabolic myopathies or disorders of neuromuscular transmission [87, 88]. From a clinical perspective, fatigue is difficulty with initiation or maintenance of voluntary activities and may include muscular, neurological, emotional, and cognitive components [88]. In the present work, fatigue refers to self-percepted fatigue [88]. Another term of fatigue is excessive tiredness. To discriminate excessive and normal tiredness, generic multi-item measures, such as Fatigue Severity Scale (FSS) [89], have been constructed with cut-offs derived from general population studies. In a previous Norwegian population study, fatigue prevalence based on the original FSS cut-off of ≥ 4 on a 1-7 scale (7 = maximal level of fatigue) was 47.5% compared to 23.1% with a cut-off of ≥ 5, suggesting that the latter cut-off was more appropriate [90].

1.6.2 Role, predictors, and perpetuating factors

Fatigue is commonly increased in populations with chronic health conditions, including muscle diseases, and reported as a major disabling symptom [80, 91]. Knowledge of predisposing, precipitating, and perpetuating factors is key to prevention and management. A meta-analysis indicated that only 11% of the fatigue variance among people with chronic

diseases is diagnosis-related, whereas 44% is related to generic factors, including female gender, young age, motivational or concentration difficulties, poor physical functioning, low levels of physical activity, pain, sleep disturbance, and a low perceived self-control of fatigue [91]. A prospective observational study on NMD suggested that low levels of physical activity, poor sleep, and pain are perpetuating factors of fatigue and that muscle weakness contributes to fatigue indirectly by limiting the level of physical activity [92]. In accordance with this explanatory model, interventional studies with exercise therapy or cognitive behavioral therapy (CBT) with mixed interventions (e.g., concerning pain, sleep, social participation, dysfunctional thoughts, and promotion of an active lifestyle yet energy saving) have been performed on NMD and showed efficacy [93-95]. In facioscapulohumeral muscular dystrophy, both aerobic exercise and CBT have shown efficacy on fatigue [96], and muscle MRI has even indicated disease-modifying effects [97], whereas an earlier study concluded that strength training did not relieve fatigue [98]. In post-polio myelitis, neither aerobic exercise or CBT proved efficacy on fatigue for unknown reasons [93]. Proposed mechanisms of the benefits of exercise therapy or CBT observed are epigenetic and anti-inflammatory effects [93].

1.7 Sleep disturbance and sleep disorders

1.7.1 Definition and classification

It is well established that sleep is vital for biological health and HRQoL [99]. While sleep disturbance is an unspecific term of persistent sleep abnormalities, subjectively or objectively, sleep disorders are diagnostic entities. The International Classification of Sleep Disorders (ICSD), developed by the American Academy of Sleep Medicine (AASM) in cooperation with international sleep societies, is the most widely used classification system for sleep disorders. The most recent edition (ICSD-3, AASM 2014) includes seven main categories of sleep disorders [100]:

- Insomnia
- Sleep-related breathing disorders
- Central disorders of hypersomnolence
- Circadian rhythm sleep-wake disorders
- Parasomnias
- Sleep-related movement disorders
- Other sleep disorders

The fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5), published by the American Psychiatric Association, classifies sleep disorders in a similar manner [101].

Studies have shown that NMD is associated with both insomnia and sleep-related breathing disorders (sleep-disordered breathing).

1.7.2 Insomnia

In ICSD-3, insomnia is defined as persistent difficulty with initiation or maintaining sleep despite adequate opportunity and circumstances for sleep, and there must be associated daytime impairment or distress [100]. Chronic insomnia disorder requires symptoms at least three days per week for at least three months [100]. ICSD-3 does not discriminate between primary and secondary (or comorbid) insomnia, but insomnia should not be diagnosed when the insomnia symptoms are better explained by another sleep disorder [100]. Consequently, a definite diagnosis of chronic insomnia disorder requires a clinical interview and sometimes additional objective assessments, such as polysomnography (PSG) in case of clinical suspicion of e.g., periodic limb movement disorder or sleep apnea, and actigraphy in case of clinical suspicion of circadian rhythm disorder [102].

Insomnia is recognized as the most common sleep disorder [99] and tends to be more prevalent in the female population [102]. Norwegian studies using the Bergen Insomnia Scale (BIS) reported a general prevalence of insomnia of 20% (females 24%-25%, males 15%-16%) [103, 104] and 54% in patients visiting their general practitioner [105]. Insomnia is related to mental, medical, and physical comorbidity, and other categories of sleep disorders [102, 106]. Some relationships may be bi-directional, as in comorbid insomnia and sleep

apnea (COMISA) [107]. Suggested predisposing factors of insomnia include genetic factors, physiological, cognitive, and emotional hyperarousal, and neuroticism [102]. Insomnia is commonly perpetuated by maladaptive coping strategies, such as extending time in bed or napping [102]. Insomnia is considered a heterogenous disorder, and there is ongoing research on biomarkers which may enable subtyping and personalized medicine in the future [108-110]. Current treatment of choice for chronic insomnia disorder is CBT for Insomnia (CBT-I), which usually includes psychoeducation, relaxation training, stimulus control, sleep restriction, and cognitive therapy [102].

Publications on insomnia in NMD are scarce, whereas poor subjective sleep quality and excessive daytime sleepiness (EDS) are more widely reported [111-114]. However, insomnia has been highlighted as an important sleep disorder in Duchenne muscular dystrophy given its prevalence and its potential negative impact on tolerance to noninvasive ventilation, and on HRQoL [115]. Additionally, a study on slowly progressive muscular dystrophies indicated a relationship between insomnia and fatigue [116].

1.7.3 Sleep-disordered breathing

Sleep-disordered breathing (SDB) is characterized by abnormal respiration during sleep and comprise four main groups [100]:

- Central sleep apnea syndromes
- Obstructive sleep apnea (OSA) disorders
- Sleep-related hypoventilation disorders
- Sleep-related hypoxemia disorder

Sleep apnea requires at the minimum an apnea-hypopnea index (AHI) of 5 events per hour. Additional criteria apply to the various phenotypes. OSA requires either an obstructive AHI ≥ 15 or an AHI ≥ 5 with predominantly obstructive events and associated signs or symptoms or at least one of the specified comorbidities: hypertension, coronary artery disease, stroke, congestive heart failure, atrial fibrillation, diabetes mellitus, cognitive dysfunction, or mood disorder [100]. The definition of sleep-related hypoventilation is based on carbon dioxide (CO_2) levels, since hypoxemia is less specific for hypoventilation. Noninvasive transcutaneous or end-tidal CO_2 monitoring has become recommended proxy methods for arterial CO_2 in diagnostics [100, 117]. Sleep-related hypoxemia is defined by oxygen

saturation ≤ 88% for > 5 consecutive minutes in the absence of sleep-related hypoventilation or CO2 measurement [100].

Muscle diseases may predispose to all the four groups of SDB through restrictive breathing (due to muscle weakness, chest wall stiffness, or scoliosis), upper airway involvement (due to hypotonia or macroglossia), or cardiomyopathy [118, 119]. Additionally, general risk factors for OSA apply [120], and complex relationships between the different SDB phenotypes may exist [121, 122]. SDB is associated with increased morbidity and mortality in NMD [118]. Management usually includes Positive Airway Pressure (PAP) therapy, such as CPAP (provides one level of pressure continuously) in isolated sleep apnea, and BiPAP (facilitates both the inspiratory and expiratory phase by providing two different pressure levels: one during inhalation and a lower during exhalation). Additionally, relevant comorbidities such as obesity [123], cardiac failure [124], and insomnia [107] should also be treated.

Regarding sleep-related hypoventilation or hypoxemia, studies on NMD show that the prevalence is highly dependent on diagnostic criteria [125-128]. In Duchenne muscular dystrophy, where respiratory involvement is relatively extensively studied, disease-specific treatment criteria of SDB have been established, but comparative trials remain [128]. In Duchenne muscular dystrophy, current follow-up guidelines include PSG and nocturnal PCO2 monitoring annually in non-ambulatory or symptomatic patients [115]. While several LGMD subtypes are associated with respiratory involvement and ventilatory needs [40, 129], respiratory guidelines have still not been developed for LGMD.

2 Aims and objectives

2.1 Aims

This work aimed to update and extend knowledge of the epidemiology of LGMDR9 in Norway, expand knowledge of natural history of LGMDR9, and to investigate relationships with HRQoL and sleep in the Norwegian LGMDR9 population. The overall goal is to improve patient outcomes by providing fundaments for optimizing clinical management, and by providing data to translational research, clinical trials, and decision forums that assist in the development and implementation of beneficial treatment.

2.2 Objectives of Paper I

The epidemiological part of the study included investigation of the minimum prevalence of LGMDR9 in Norway overall and in the adult and pediatric population separately, the geographical distribution, sex distribution among *FKRP* c.826C>A homozygotes, genetic spectrum, and the carrier frequency of the common *FKRP* c.826C>A variant and of any *FKRP*-related disease-causing variant (i.e., general carrier frequency), respectively, in the general population. We expected that the diagnostic coverage had increased since the previous Norwegian prevalence study in 2009, when the diagnostic test was relatively new. We also expected the prevalence to be higher in the adult compared to the pediatric population due to a relatively late onset, slowly progressive nature, and a long life expectancy, in general. Based on the Norwegian regional epidemiological studies previously mentioned (Sect. 1.2), we expected a relatively higher prevalence in Northern Norway and a relatively lower one in Vestland county. Lastly, we expected a comparable number of female and male *FKRP* c.826C>A homozygotes. Nevertheless, if one sex was less affected, this sex could be underrepresented. We also examined the proportion of *FKRP* c.826C>A homozygotes that are homozygous for the SNP, *FKRP* c.135C>T (Sect. 1.2). A 100% association also in the Norwegian sample would consolidate the theory that *FKRP* c.826C>A is a founder variant and unlikely to occur spontaneously.

Concerning natural history, we assessed clinical features, genotype-phenotype relationships, inter-sibling variation, temporal relationships, and correlations. While multiple natural history studies on LGMDR9 have been performed previously (Sect. 1.3), our main objective was to study patterns and variation in a large sample of *FKRP* c.826C>A homozygotes, i.e., genotypically homogeneous individuals. We examined temporal relationships and correlates of the three endpoints wheelchair dependency, PAP therapy, and cardiomyopathy. Potential predictors assessed were age at disease onset, disease duration, age, and sex, and additionally level of ambulation for cardiomyopathy and both level of ambulation and cardiomyopathy for PAP therapy.

2.3 Objectives of Paper II

We studied changes in HRQoL in our LGMDR9 cohort over 14 months. Furthermore, to identify possible aspects of HRQoL, risk groups, and specific symptoms in need of an increased attention, we compared HRQoL to a reference population and examined

relationships between the perceived burden of disease and genotype, sociodemographic variables, disease duration, disease involvement, LGMDR9-related symptoms, and subjective quality of sleep.

We did not expect any significant changes over the short time span of 14 months considering the slowly progressive nature of the disease, in general. Based on previous studies on other samples with muscle disease, we expected that both physical, mental, and social aspects of HRQoL would be affected. From natural history (Paper I), it was most intuitive that *FKRP* c.826C>A compound heterozygotes would report relatively poorer HRQoL than *FKRP* c.826C>A homozygotes, and correspondingly for *FKRP* c.826C>A homozygous females compared to *FKRP* c.826C>A homozygous males. Additionally, there was a tendency that females reported poorer HRQoL than males in the reference population. Concerning social factors, we expected levels of higher education to correlate positively with HRQoL, since this a well-known association. Living alone could relate positively to HRQoL due to higher levels of independence or the opposite due to e.g., dependency of assistance from outside the family, an increased household burden, or loneliness. Full long-term sickness absence was expected to relate negatively to HRQoL due to more advanced disease and the lack of the mental and social health benefits of working, e.g., development of skills, having a daily structure, a sense of purpose and identity, and social participation. Correlations between aspects of disease burden and age or disease duration likely reflect relationships with the progression of disease. Lack of correlation could reflect aspects that are more influenced by personal factors than disease severity. Such aspects of burden may need particular awareness at an early stage of disease. Tentative positive predictors of disease burden were advancing age, being a female, perceived muscle weakness, myalgia, fatigue, dysphagia, poor/lost ambulation, cardiomyopathy, PAP therapy, sleep disturbance, and the lack of higher education.

Further, we investigated the prevalence of fatigue and whether fatigue in LGMDR9 correlates with age, sex, perceived muscle weakness, myalgia, negative emotions, or sleep disturbance. Being a female was assumed to be a potential risk factor based on the aforementioned meta-analysis of fatigue in chronic diseases (Sect. 1.6.2). Additionally, there was a tendency that females in the reference population reported relatively poorer scores than males on the SF-36 subscale of vitality [130, 131]. Finally, we examined whether subjective sleep quality correlates with age, sex, perceived muscle weakness, myalgia, negative emotions, wheelchair dependency, or PAP therapy. Being a female could be a risk factor since insomnia, as

mentioned, is the most common sleep disorder in the general population and was found to be relatively more prevalent in females than males (Sect. 1.7.2).

2.4 Objectives of Paper III

We investigated the occurrence of insomnia and unrecognized or untreated SDB and their relationships with demographic and clinical variables and aspects of HRQoL, especially fatigue. Additionally, we assessed whether fatigue is associated with impaired pulmonary function, since respiratory muscle weakness and chest wall alterations, such as scoliosis and stiffness, tend to increase the work of breathing.

We expected that insomnia would be prevalent since insomnia is associated with somatic and mental health conditions and other sleep disorders. Previously, we had found that the study participants had impaired physical and mental HRQoL (Paper II), moreover that a high proportion of the study participants have nocturnal PAP therapy and thus underlying SDB (Paper I). Furthermore, we assessed whether fatigue is associated with insomnia. A positive relationship would suggest that insomnia could potentially be a target in the treatment of fatigue. We also hypothesized that insomnia is associated with advancing age (due to increased burden of disease (Paper II)), being a female (due to increased burden of disease (Paper II) and findings in the reference population (Sect. 1.7.2)), PAP therapy (due to discomfort or suboptimal treatment), wheelchair dependency (due to mobility issues), and impaired HRQoL.

We further hypothesized that SDB is underrecognized due to the lack of respiratory guidelines in LGMD, and that this may be a significant contributing factor to fatigue. Since EDS is commonly associated with SDB, a high prevalence of EDS may be an indication that SDB is underrecognized or undertreated. The rate and severity of SDB among patients without PAP therapy would also give us an indication of whether SDB is underrecognized or recognized but untreated when compared to diagnostic guidelines. Association between fatigue and EDS or nocturnal indicators of SDB would suggest that SDB may be a significant contributing factor to fatigue. Additionally, we examined whether the AHI correlates with respiratory HRQoL, hence a severity biomarker. Based on background knowledge, tentative positive predictors of sleep apnea were age, being a male, Body Mass Index, cardiac failure, pulmonary dysfunction, and oropharyngeal involvement (macroglossia, dysphagia, or dysarthria).

3 Materials and methods

3.1 Recruitment and data collection

The epidemiological study included all living subjects with genetically confirmed LGMDR9 residing in Norway as of January 1st 2021. The individuals were primarily identified through diagnostic patient registries at The Medical Genetics Departments of the University Hospital of North Norway HF (UNN) and Telemark Hospital Trust (THT) and the Department of Neurology at Haukeland University Hospital (HUS), which together comprised all the relevant diagnostic entities. Most of the individuals with LGMDR9 were diagnosed at UNN. Personal identity information on those few individuals diagnosed at HUS and THT were received by phone, and was required to get the full overview of number of families and ensure that none were counted twice. Secondary, the Norwegian Registry of Hereditary and Congenital Neuromuscular Disorders was searched for unidentified individuals with LGMDR9. Additionally, the Global FKRP Registry informed their Norwegian registrants about the study and where to get further information or an invitation letter. This also could assist in capturing unidentified individuals.

All individuals with genetically verified LGMDR9 were invited to participate in the cohort study by the respective institutions. The patients received a letter with information, a consent form, a study-specific questionnaire, and a reply envelope to NMK at UNN. There were no exclusion criteria. Participation included completing the attached questionnaire and consent to the retrieval of relevant extracts from patient records at the specialist centers/hospitals. Adults (≥ 16 years old) were also invited to the clinical part of the study, which consisted of a 2-day stay at UNN with a battery of tests and a planned follow-up after 2 years, and received the questionnaires of HRQoL, sleep, and fatigue.

An overview of the recruitment and data collection is provided in Figure 3-1. The recruitment started in June 2019. The surveys were conducted during the COVID-19 pandemic, but in relatively normal COVID-19 free periods: HRQoL in June 2020, February 2021, and August 2021, and the sleep survey in March 2021. Baseline clinical inclusion took place in the period January 2020 to November 2021, but intermittently according to COVID-19 restrictions. Due to several dropouts of participants who had originally signed up for clinical participation, a new invitation letter to clinical participation was sent out autumn 2021.

Two reminders were sent after the first invitation letter. One reminder was sent for each survey after about 4 weeks. The surveys were administered by regular mail, and SMS was used for reminders. Patient who had consented to participate in the clinical study were contacted by phone to make appointments. Patients without PAP therapy were invited to also undergo PSG during their visit at UNN.

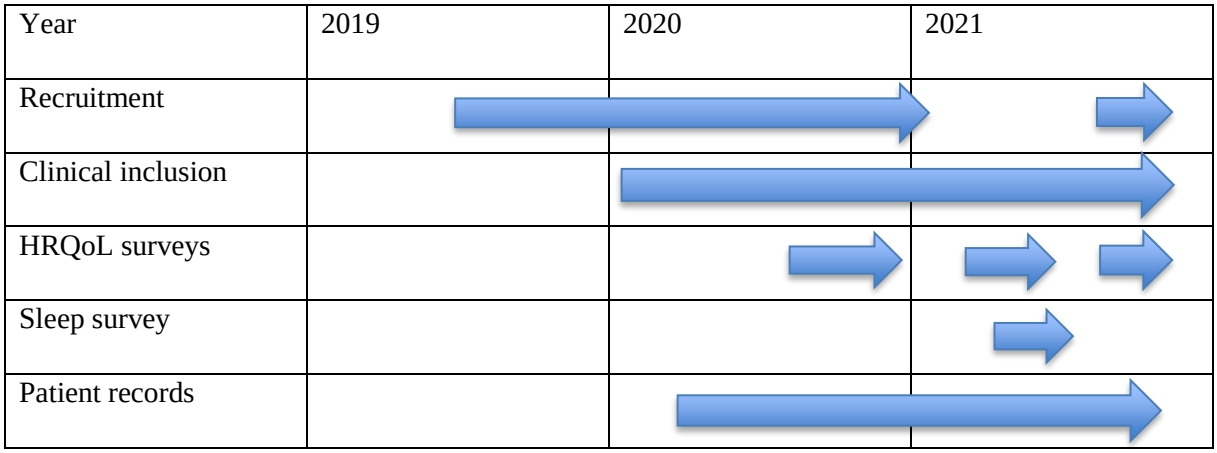

Figure 3-1 Temporal overview of the recruitment and data collection

3.2 Procedures

For the epidemiological analyses, number of individuals and sib-ships with LGMDR9, genotypes, age, sex, and county of residence were recorded. Population numbers for calculation of prevalence and carrier frequencies were received from Statistics Norway [132]. Presence of the SNP, *FKRP* c.135C>T, was assessed in *FKRP* c.826C>A homozygotes who were genetically confirmed at UNN. The observed homozygote frequency of the SNP *FKRP* c.135C>T allele among Europeans (non-Finnish) is 1% [26].

Patient notes were retrieved on consenting participants. The clinical participants were included one-by-one. The program for the clinical visit is shown in Table 3.1.

Natural history data (Paper I) were obtained from the patient records, the study-specific questionnaire, and from the neurological examination of the clinical participants. The participants were divided into four subgroups by genotype (*FKRP* [c.826C>A/c.826C>A] vs other genotypes) and sex. Clinical characteristics and natural history were presented for each subgroup, and additionally at individual level for *FKRP* c.826C>A homozygous sib-ships and those with “other genotype”. Temporal relationships were analyzed and compared between

FKRP c.826C>A homozygous males and females and between the two genotype subgroups. Correlation analyses of natural history were performed only in *FKRP* c.826C>A homozygotes to eliminate noise from genotype effects.

Paper II included all the three HRQoL surveys (Sect. 3.3.1) and two instruments from the sleep survey» (Sect. 3.3.2): the Fatigue Severity Scale (FSS) and the Pittsburgh Sleep Quality Index (PSQI). Background characteristics were based on the study-specific questionnaire, but disease onset primarily on the patient records to avoid recall bias. The respondents were divided into subgroups by genotype (*FKRP* c.826C>A homozygotes and *FKRP* c.826C>A compound heterozygotes) and sex.

Paper III included the second HRQoL survey and all the instruments in the «sleep survey» (Sect. 3.3.2) except PSQI. The results of the sleep survey were presented for respondents with and without PAP therapy, separately. Background data were based on the study-specific questionnaire. Paper III also included a PSG study of clinical participants without PAP therapy, and additional assessments of the PSG candidates. Those data were presented for males and females, respectively. Genotype was not considered. PSG candidates were instructed to avoid sedative and central stimulant drugs in the last 2 weeks before sleep registration. From neurological examination, we included assessment of macroglossia, dysarthria, and self-reported dysphagia. From echocardiography, we reported left ventricular ejection fraction and classified it as < 50% (abnormal) or ≥ 50% (normal).

Data were collected and managed using REDCap[1] electronic data capture tools hosted at UNN [133, 134], optically readable instruments, and dedicated SPSS-compatible software for echocardiography and PSG, respectively.

[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

Table 3-1 Program for the clinical visit

Day 0	Check in at the patient hotel
Day 1	• Blood sample and capillary blood test at the lab (7.30 a.m.) • Body Mass Index [b], neck/hip/waist circumference • Electrocardiogram • Peak Cough Flow • Self-reporting questionnaires: VAS fatigue, VAS pain, FSS [b], and respiratory questionnaire [b] • Muscle ultrasound • Motor tests • Mounting of PSG (15 p.m.) [b]
Day 2	• Detachment of PSG (7.30 a.m.) [b] • Pulmonary function tests: spirometry [b], MIP [b], MEP, plethysmography • Echocardiography [b] • Neurological examination [a, b] • Cognitive test • Motor tests [b]

[a] Included in Paper I, [b] Included in Paper III. Abbreviations: VAS = Visual Analogue Scale, FSS = Fatigue Severity Scale, PSG = polysomnography, MIP = Maximal Inspiratory Pressure, MEP = Maximal Expiratory Pressure

3.3 Self-report measures

Study-specific questionnaire: Appendix.

3.3.1 HRQoL

The 36-Item Short-Form Health Survey (SF-36) version 1 [78] is a generic profile-based HRQoL that measures nine dimensions of HRQoL: physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotions, mental health, and change in health over the last year. The Norwegian version and Norwegian reference data were applied [130, 131].

The Individualized Neuromuscular Quality of Life questionnaire (INQoL)[2] version 2.0 [76] is a disease-specific HRQoL that contains seven symptom domains (i.e., muscle weakness, myalgia, fatigue, myotonia, diplopia, ptosis, and dysphagia), five life domains (i.e., activities related to daily living/ leisure/ work, independence, social relationships, emotions,

[2] INQoL © 2005 M R Rose & King's College NHS-Trust. All rights reserved.

and body image), and two treatment domains (i.e., perceived and expected treatment effects). Each domain contains items on health status severity, impact severity, and impact importance, which are all included in the respective domain scores. Additionally, the items on impact severity and impact importance (but not health status) of the five life domains are aggregated into the INQoL index as a measure of disease burden, i.e. the degree of negative impact on HRQoL. The impact of a given health status is individual by depending on personal circumstances, values, and references, which the name of the instrument reflects. The domains of myotonia, diplopia, ptosis, and treatment were not found relevant in the current project, hence not included in the analyses. We used the Norwegian version of INQoL It had been translated by NMK, using linguistic validation methodology with forward-backward translation, and tested on 100 outpatients (with 70 respondents) at NMK with various NMD proving satisfactory internal validity and concurrent validity with the SF-36 (unpublished data).

3.3.2 Fatigue and sleep

Fatigue Severity Scale (FSS)[3] [89] is a generic measure where the mean of nine items scores (range 1 to 7) indicates the fatigue severity. The Norwegian version was applied. Cut-off for significant fatigue was set to FSS ≥ 5 as recommended in a Norwegian validation study [90].

Pittsburgh Sleep Quality Index (PSQI)[4] [135] provides a global score of subjective sleep quality (range 0 to 21) with a cut-off score of > 5 indicating significant sleep disturbance. The Norwegian version was applied [136].

Epworth Sleepiness Scale (ESS)[5] [137] measures daytime sleepiness by assessing the chance of dozing off (range 0-3) in eight situations. A sum score > 10 indicates EDS. The Norwegian version was applied [138].

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[3] © 1985 Lauren B. Krupp. Reproduced with permission from the author.

[4] © 1989, University of Pittsburgh. Developed by Buysse,D.J., Reynolds,C.F., Monk,T.H., Berman,S.R., and Kupfer,D.J. of the University of Pittsburgh using National Institute of Mental Health Funding. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ: Psychiatry Research, 28:193-213, 1989. All rights reserved. Translated in 2006, by Mapi Linguistic Validation under License and distributed by Mapi Research Trust under license.

[5] ESS © MW Johns 1990-1997. To Norwegian with permission from the rights holder.

Bergen Insomnia Scale (BIS) is a Norwegian instrument that can be used to assess whether chronic insomnia is present or not, and the severity of insomnia symptoms. The original BIS was based on the criteria for chronic insomnia of the DSM, 4th edition (DSM-4) [139], but later adjusted to the criteria of DSM-5/ ICSD-3 [103]. The minimum BIS criterion for insomnia is a score of ≥ 3 on both one nighttime item (sleep onset latency, excessive wake time after sleep onset, or early morning awakening) and one daytime item (tiredness or dissatisfaction with sleep). The insomnia severity is measured by the sum score of all the nighttime and daytime items and an item of nonrestorative sleep.

QoL questions for patients on home mechanical ventilation (here: «respiratory questionnaire») assesses five SDB-related HRQoL items (i.e., several awakenings, nocturnal dyspnea, nonrestorative sleep, morning headache, and daytime tiredness). The questions origin from a Swedish stress research program [140] and were applied as a condition-specific HRQoL measure on patients with chronic alveolar hypoventilation in research [141, 142] and in the Swedish [143] and the Norwegian national register for patients on home mechanical ventilation [144]. The Norwegian version was applied and deviated slightly from the Swedish version. Only the Swedish version had been validated.

3.4 Polysomnography and PtcCO2 monitoring

SOMNOscreen equipment and Domino version 2.7.0 software (Somnomedics, Randersacker, Germany) were used for PSG. A pressure flow oral/nasal sensor was used for measurement of air flow. PSG recording and scoring were performed in accordance with the AASM guidelines, 2017 [145]. The scoring was divided between two neurophysiologists at UNN. For nocturnal PtcCO2 monitoring, we used SenTec Digital Monitoring System (SenTec AG, Therwil, Switzerland). The CO2 sensor was placed on the forehead to minimize the influence from external pressure or temperature and detachment due to movements. The CO2 measurements were read automatically, but also checked by the PhD candidate for artifacts and events of hypoventilation.

We applied the 3% criterion for desaturation [145]. Apneas were classified as obstructive, central, and mixed [145]. Hypopneas were not classified since they are less specific and thus more prone to misclassification, especially in NMD were central or obstructive events may be confused with or compounded by diaphragmatic events. In the present study (Paper III), SA

was defined as AHI ≥ 5 per hour sleep. We used the well-established classification of SA severity: AHI 5 to 15 = light, AHI 15 to 30 = moderate, and AHI 30 or more = severe [145]. ICSD-3 criteria for sleep-related hypoxemia (Sect. 1.7.3) were applied, and AASM criteria for sleep-related hypoventilation (ie., an increase in PtcCO2 to a value > 55 mmHg (7.33 kPa) for ≥ 10 minutes and/or ≥ 10 mmHg (1.33 kPa) increase in PtcCO2 during sleep in comparison to awake supine value to a value exceeding 50 mmHg (6.67 kPa) for ≥ 10 minutes) [117].

To assess relationships with fatigue, the following variables were used (Paper III): Total sleep time, amount rapid eye movement (REM) sleep (minutes and percent of total sleep time), amount Non-REM sleep stage 3 (i.e., deep sleep) (minutes and percent of total sleep time), AHI, ODI, microarousal index, mean oxygen saturation, and mean PtcCO2.

3.5 Pulmonary function tests

Spirometry was performed according to The American Thoracic Society and the European Respiratory Society technical guidelines 2019 [146] and performed both in the standard sitting position and supine. Whereas lung volumes are compromised in the horizontal compared to the vertical position physiologically [119], a large relative drop in FVC is an indicator of diaphragm weakness [147]. MIP was performed in a sitting position, and the best of at least three repeated measurements was recorded as MIP percent predicted (MIP%). Since the diaphragm is the main inspiratory muscle, MIP% was applied as a second indicator of diaphragm weakness. Diaphragm weakness was relevant in relation to SDB due to REM sleep atonia, i.e., when the respiratory muscles (and most other skeletal muscles) except the diaphragm are more or less physiologically paralyzed [121]. Inspiratory strength was considered relevant in relation to fatigue, since inspiration is the active phase of ventilation, and thus the main component of the work of breathing (at least in non-obstructive disorders). Therefore, maximal expiratory strength was not considered relevant for the purpose of the study.

The following pulmonary variables were used (Paper III): FVC sitting and supine percent predicted based on reference equations in the standard position [148], the relative FVC drop from sitting to supine posture, and MIP% [149].

3.6 The 32-item Motor Function Measure (MFM32)

MFM32 is a clinician-reported scale of gross motor function in individuals with NMD [150] and was peformed by MFM32-certified physiotherapists at NMK. The scale provides a total score and three subdomain scores: 1) Standing and transfers, 2) Axial and proximal motor function, and 3) Distal motor function. It was used to explore correlations with fatigue in Paper III.

3.7 Statistical analyses

IBM SPSS 27 was used for Paper I and II and IBM SPSS 28 for Paper III.

In Paper I, group differences for continuous outcome variables were assessed using independent t-test on normally distributed data, and Mann-Whitney U-test on non-normally distributed data. In Paper II and III, independent t-test with bootstrapping (5000 resamplings), which handles both distributions, was applied. Kruskal-Wallis test was used if multiple subgroups. Group differences for categoric outcome variables were examined with chi-square test, or Fisher exact test with mid-p correction when the assumptions for chi-square test were not met. Bivariate correlations were examined with Pearson or Spearman rank correlation according to distribution of data (normal vs non-normal). Multivariate regression (linear, logistic, Cox, or generalized linear mixed model, as appropriate) was used to control for confounding effects. Among predictor variables of interest, p-value in simple regression was used to select the most relevant variables for the multivariate regression model. In Paper II and III, the backward-elimination option on linear multiple regression was utilized to define the simplest model that explained the data, but the initial model was also presented. Residuals were inspected for non-normality, heteroscedasticity, and influential cases except when special methods were applied (i.e., bootstrapping or generalized linear mixed model with robust estimation of the standard deviations). All tests were performed with a two-sided alpha level of 0.05.

Paper I: Allele and carrier frequencies in the general population were estimated using the Hardy-Weinberg equation, which states that allele frequencies of an autosomal gene locus in a population remain constant in a large population over time if there is random mating with respect to the locus and no natural selection, mutations, gene flow (migration), or genetic drift

(occurs in small population when individuals with the specific allele by chance reproduce more or less than the others) [151]. For a population in equilibrium the following applies:

$q^2 + p^2 + 2pq = 1$ and $p + q = 1$

q = allele frequency, p = frequency of not having the allele, q^2 = frequency of recessive subjects, p^2 = frequency of subjects not having the allele at all, and 2pq = carrier frequency

By calculating the prevalence of *FKRP* c.826C>A homozygotes in the Norwegian population (q^2), the c.826C>A carrier frequency (2pq) in the same population could then be estimated. Similarly, by including all LGMDR9 subjects in the prevalence, the general LGMDR9 carrier frequency in the Norwegian population could be estimated.

Two populations with the same carrier frequency and in equilibrium with respect to the allele may show different disease prevalence by chance. To evaluate whether the difference between the lowest and the highest prevalence at county level occurred by chance, the random variation for each of them was estimated by a 95% confidence interval. For this we used the Wilson interval score for proportions, which is considered robust, performs well across all values of observed proportions and sample sizes, and always computes intervals between 0 and 1 [152].

In the natural history study, distribution in age of onset was analyzed by histogram and two-step cluster-analysis to explore possible natural groupings. Time to endpoints with censored observations (i.e., wheelchair dependency, detected cardiomyopathy, and initiation of PAP therapy) were presented with Kaplan-Meyer survival (time-to-event) curves with age as time scale. Fifty percent accumulated probabilities were estimated when applicable. Survival curves were compared by Log-rank test (between *FKRP* c.826C>A homozygous males and females) or Cox-regression (between genotype subgroups to control for sex) with estimation of Hazard ratios. Hazard ratio compares the rate of an event between two subgroups. To identify potential predictors of outcome variables, multiple regression was applied. Level of ambulation as dependent continuous variable (the sum score of three items: maximal walking distance on flat ground, stair climbing, and the ability to run) was assessed by multiple linear regression with regression coefficients (beta) as effect size. Beta describes the amount of change in the outcome variable for every 1-unit of increase in the predictor variable. Wheelchair dependency, cardiomyopathy, and PAP therapy as binary dependent variables

were assessed by multivariate binomial logistic regression with estimation of odds ratios. Odds ratio compares the odds of an event (probability of occurring versus not occurring) between two subgroups or for 1-unit of increase in the predictor variable. We also performed a post-hoc analysis to assess the significance if corrected for multiple subgroup comparisons (a peer-review suggestion). The Holm-Bonferroni method was chosen, which is less conservative than the commonly used Bonferroni. False negative findings were considered the most undesirable in the study of natural history, since it was exploratory and not constructed to make evidence.

Paper II: For comparisons of SF-36 data between the target and reference population, independent t-test was found acceptable given the sample sizes. For comparisons between the two genotype subgroups, multiple linear regression with bootstrapping was applied to adjust for age and sex. Measurement of longitudinal change between baseline and 14 months from baseline was performed by paired t-test with bootstrapping in *FKRP* c.826C>A homozygotes, and with Wilcoxon paired signed rank test in *FKRP* c.826C>A compound heterozygotes due to low sample size. For inclusion of all three repeated measurements, linear mixed models were applied, which handle missing data and data dependencies. In prospective repeated measurements, a high rank order stability is desired, i.e., low levels of random variability across time. The rank-order stability was evaluated by the intraclass correlation coefficient which was estimated as the ratio between inter-subject variance and total variance (inter-subject + intra-subject variance) in a linear mixed model without fixed effects. In order to both assess longitudinal change and identify predictors of the two outcome variables *Perceived muscle weakness* and the *INQoL index*, generalized linear mixed model regression with robust estimation of the standard errors was performed. The Bayesian Information Criterion value was used for the decision of covariance structure of the residual matrix (the smaller, the more preferable).

Due to the exploratory design and limited power, correcting for multiple testing was not considered appropriate [153]. With alpha level 0.05, we accept that at least 1/20 tests are expected to be false positive.

Paper III: Bivariate correlations were examined with scatter plots considering the risk of outlier bias at low sample size. Multiple regression of the AHI was performed. Based on

simple regression, an ejection fraction < 50% was found to be a relevant predictor variable in contrast to pulmonary function and oropharyngeal involvement. Both age, sex, and Body Mass Index were considered necessary to include due to potential confounding and mediator effects (Figure 3-2): Findings in Paper I suggested that males were more predisposed to cardiomyopathy. In the general population, cardiac failure is associated with advancing age, and sleep apnea with advancing age, obesity, and being a male. The AHI variable was square-root transformed to meet the assumptions for the residuals. Since the results showed similar findings as the original model, the original model was kept to show interpretable effects. Correction for multiple testing was not used for the same reason as in Paper II.

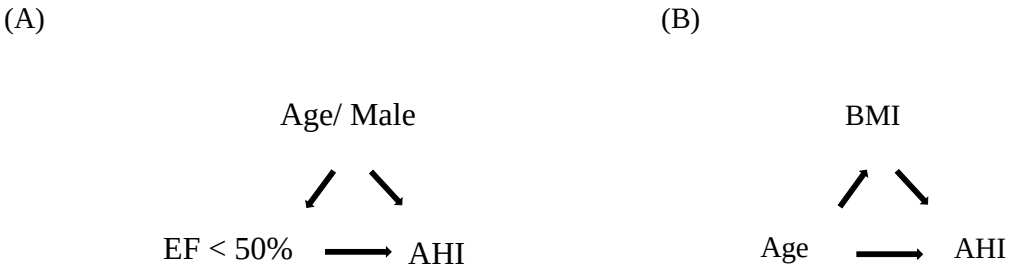

Figure 3-2 Theoretical models of potential predictors, confounders, and mediators with the apnea-hypopnea index as the outcome variable: (A) Left ventricular ejection fraction (EF) <50% as the predictor variable. Age and sex as confounders. (B) Age as a predictor variable. Body Mass Index (BMI) as a mediator.

A summary of material and methods are presented in Table 3-2.

Table 3-2 Summary of methods and material

	Paper I	**Paper II**	**Paper III**
Main title	Epidemiology and natural history in 101 subjects with LGMDR9	HRQoL in LGMDR9	Insomnia and sleep-disordered breathing in LGMDR9
Design	Observational, retrospective and cross-sectional	Observational, cross-sectional and prospective with repeats	Observational, cross-sectional
Statistical methods	Simple group comparison, correlation, two-step cluster analysis, survival analyses, Cox regression, multiple linear, logistic regression	Simple group comparison, paired tests, correlation, multiple linear regression, generalized linear mixed model	Simple group comparison, correlation, multiple linear regression (with transformation)
Material			
Study specific questionnaire	Yes	Yes	Yes
Patient records	Yes	Yes	Yes
Self-report instruments	No	SF-36, INQoL, PSQI, FSS	SF-36, INQoL, FSS, BIS, ESS, respiratory questionnaire
Physical investigations	Clinical neurological examination	No	Clinical neurological examination, MFM32, BMI, echocardiography, spirometry, MIP, PSG, PtcCO2, capillary blood gas

Abbreviations: LGMDR9 = FKRP-related limb-girdle muscular dystrophy R9, HRQoL = health-related quality of life, SF-36 = 36-item Short Form Health Survey, INQoL = Individualized Neuromusuclar Quality of Life questionnaire, PSQI = Pittsbugh Sleep Quality Index, FSS = Fatigue Severity Scale, BIS = Bergen Insomnia Scale, ESS = Epworth Sleepiness Scale, MFM32 = The 32-item Motor Function Measure, BMI = Body Mass Index, MIP = Maximal Inspiratory Pressure, PSG = polysomnography, PtcCO2 = transcutaneous carbon dioxide tension

3.8 Ethical considerations

The study was approved by the Regional Ethics Commitee (REK) (reference number 2018/1968/REK nord) and by the Data Protection Officer at UNN. Approval of recruitment via the "Norwegian National Registry for Congenital and Hereditary Neuromuscular Disorders" and the "Global FKRP Registry" were also made by the respective registries. The Local Data Protection Officer was sought for the collection of patient notes according to local procedure. The Norwegian version of SF-36 v. 1 was freely distributed by Knowledge Centre for the Health Services, Norwegian Institute of Public Health [154]. Permission to use the remaining instruments was obtained according to need. The ESS was originally collected during the clinical participation before we became aware that ESS was licensed. Since these data could not use be used, the study was expanded with a postal survey (i.e., the "sleep survey") after REK approval and the required license agreements for ESS, FSS, and PSQI were obtained. MFM32 certification of the assessors was achieved as required.

All the participants provided written informed consent prior to the collection and use of clinical data. Participants were informed in the letter about the option for withdrawing and have their data deleted from the study, the potential discomfort of the sleep study equipment, and the potential risks of blood sampling. There were no other expected health risks. The COVID-19 pandemic was an unexpected risk factor and handled by periodic inclusion breaks in accordance with national restrictions and advices and local restrictions at UNN. REK was applied for permission before an additional recruitment of clinical participants autumn 2021.

Identity-key and digital research data were stored in separate locations assigned from the Data Protection Officer at UNN. Patient records were also stored in a locked physical archive at the NMK secretariat. Concerning the clinical part of the study, results of the clinical neurological examination, lung lab tests, echocardiography, PSG, PtcCO2 monitoring, and blood sample were also registered in the Electronical Patient Journal. Report was written to the local practitioners, and the participants were referred for further examinations or treatment if needed.

4 Results

4.1 Sample

An overview of eligible, invited, and included participants in the respective sub-studies is provided in Table 4-1. The epidemiological study (Paper I) included 153 individuals (135 adults) and should cover 100% or close to 100% of the Norwegian LGMDR9 population genetically verified. The natural history included 101 of 153 invited participants. Of these, 90 were adults and received the questionnaires of the surveys. One participant was included between the first and the second HRQoL study, and another died between the second and the third HRQoL survey. The remaining 88 adults had the opportunity to participate in all the surveys. In total, 84/90 (93%) participated in at least one HRQoL survey, and the response rate ranged 76-87%. The clinical study had 49 participants, of whom 35 did not have PAP therapy and were thus eligible for PSG. Six eligible participants did not undergo PSG, either because they declined or PSG was not offered due to practical inconvenience. Three PSG registrations were unsuccessful. The final PSG study sample comprised 26 participants.

There was a comparable number of males and females in the cohort. The sleep survey sample and the clinical sample were representative of the adults in the cohort with respect to age, sex, wheelchair dependency, and PAP therapy. Nevertheless, the PSG sample had a skewed representation by sex, consisting of 8/14 (57%) eligible females and 18/21 (86%) eligible males.

The genotype distribution in the natural history and HRQoL study, respectively, were both comparable to the national sample: The Norwegian LGMDR9 population comprised 134/153 (88%) *FKRP* c.826C>A homozygotes, 16/153 (10%) *FKRP* c.826C>A compound heterozygotes, and 3/153 (2%) that did not have the *FKRP* c.826C>A variant. The natural history sample comprised 88/101 (87%) *FKRP* c.826C>A homozygotes and 12/101 (12%) *FKRP* c.826C>A compound heterozygotes and 1/101 (1%) that did not have the *FKRP* c.826C>A allele. The HRQoL sample comprised 75/84 (89%) *FKRP* c.826C>A homozygotes and 9/54 (11%) *FKRP* c.826C>A compound heterozygotes. In Paper III, genotype was not considered.

Table 4-1 Overview of recruitment and inclusion

	Eligable/invited	Included (rate of eligable)
Prevalence study	153 (national sample)	153 (100%)
Natural history study	153 (national sample)	101 (66%)
Clinical study	135 (adult national sample)	49 (36%)
PSG study	35 (clinical participants non-PAP)	26 (74%)
HRQoL survey no. 1, 2, 3	89, 90, 89 (adults in the cohort)	73 (82%), 78 (87%), 68 (76%)
In total	90 (adults in the cohort)	84 (93%)
Sleep survey	90 (adults in the cohort)	77 (86%)

Abbreviations: PSG = polysomnography, PAP = Positive Airway Pressure therapy, HRQoL = health-related quality of life

4.2 Paper I

The epidemiological study: The overall prevalence of genetically verified LGMDR9 in Norway on 1 January 2021 was 2,84/100,000 (1.98/100,000 in in the pediatric population (< 18 years) and 3.06/100,000 in the adult population). The prevalence was highest in Northern and Central Norway and lowest in south-west Norway (Fig. 4.1, County no. 1 and 2, 3 and 4, and 5 and 6). The individual county prevalence ranged from 0.63 (95% CI: 0.24, 1.61) per 100,000 in Vestland County in south-west Norway to 8.32 (95% CI: 5.39, 12.85) per 100,000 in Nordland County in Northern Norway (Fig. 4-1, County no. 5 and 2). The *FKRP* c.826C>A homozygotes comprised a comparable number of females and males. The *FKRP* c.826C>A carrier frequency was estimated to 1/101 individuals in the general population, and the total carrier frequency to 1/94. The *FKRP* [c.135C>T/c.135C>T] genotype was detected in 100% of the *FKRP* c.826C>A homozygotes who were genetically confirmed at UNN (n = 127).

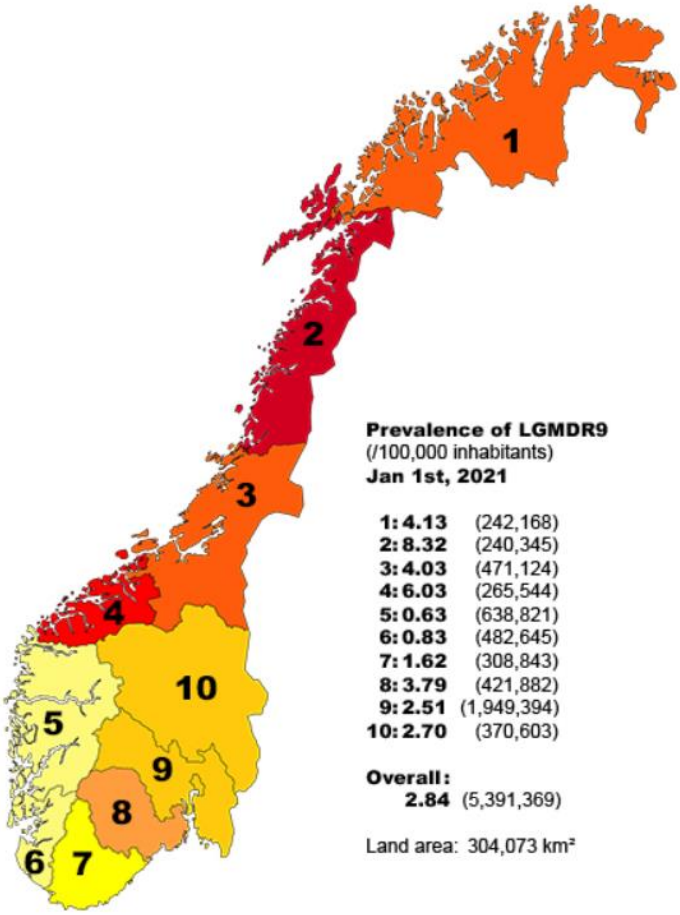

Figure 4-1 Prevalence of LGMDR9 in Norway at county level. Oslo was merged with Viken County due to a small area and similar prevalence (number 9). Colour intensity correlates with the whole number of individuals with LGMDR9 per 100,000 population. A replication of Figure 1, Paper 1.

The natural history study: Clinical characteristics were consistent with previous studies (sect. 3.1), including loss of ambulation, pseudohypertrophic calves, tendo-achilles contractures, scapular winging, macroglossia, hyperCKemia, dilated cardiomyopathy, respiratory involvement, and sometimes scoliosis and multiple contractures. Nevertheless, scapular winging was found to be relatively prevalent and exertional myalgia a common onset symptom. *FKRP* c.826C>A homozygotes showed a milder phenotype compared to the subgroup of other genotypes, in general, but clinical diversity was found in both groups, including between siblings. Wheelchair dependency preceded PAP therapy in two thirds of the PAP users. Earlier need of PAP therapy was in most cases due to SA. PAP users usually had restrictive lung function, but often combined with SA (restrictiveness only: 15/32, mixed:

10/32, SA only: 7/32). The respiratory follow-up was variable. About 20% had never seen a pulmonologist, whereas nearly all the participants had cardiac monitoring.

***FKRP c.826C>A homozygotes**:* Demonstrated a bimodal distribution in age of onset with a predominant early onset cluster (75%) with a peak at 8-9 years, and an adult-onset cluster (25%) with a peak in the mid-20s. Median time from symptom onset to diagnostic work-up was 10 years. Females had a significantly increased accumulated probability of wheelchair dependency and initiation of PAP therapy in comparison to males, whereas the opposite tendency was found for cardiomyopathy. The latter was not significant if corrected for multiple comparisons. The predicted age of 50% probability of wheelchair dependency in females was 49 (95% CI: 37, 61) years, and of PAP 52 (95% CI: 44, 60) years, whereas males did not reach the 50% level. The predicted 50% probability of being diagnosed with cardiomyopathy was by age 55 years (95% CI: 44, 66) in males, whereas females did not cross the 50% level. Multivariate regression showed that level of ambulation or wheelchair dependency was predicted by sex (females worse) and disease duration. PAP therapy was predicted by advancing age and inversely by ambulatory level. Cardiomyopathy was predicted by sex (males worse) and was unrelated to ambulatory level, age, and disease duration. None of the endpoints were related to age of onset.

***Participants with other genotypes*:** Showed at group level earlier onset and clinical endpoints compared to c.826C>A homozygotes. The predicted 50% probability of wheelchair dependency was by age 20 (95% CI: 7, 33) years and of being diagnosed with cardiomyopathy 31 (95% CI: 24, 38) years, whereas the 50% cumulative probability of PAP therapy could not be estimated. The genotype-related difference in age of onset was significant, but not when corrected for multiple comparisons. The difference in accumulated probability of wheelchair dependency and initiation of PAP therapy, respectively, was significant, also when corrected for multiple comparisons. The accumulated probability of cardiomyopathy was non-different.

4.3 Paper II

Prospective analyses: The three cross-sectional measurements were comparable. Most variation was found in the aspects of pain, fatigue, and vitality. The intraclass correlation coefficients indicated satisfactory rank-order stability for most of the HRQoL subscales, but

generally better for those of INQoL than SF-36. Between baseline and 14 months, *FKRP* c.826C>A homozygotes showed worsening of perceived muscle weakness and overall burden. In parallel, compound heterozygotes showed no significant changes but a tendency towards worsening of weakness and a paradoxical reduction of burden.

Health areas and subgroups of impaired HRQoL: All aspects of SF-36 were significantly impaired except mental health in males (borderline significant) and pain in both males and females. All aspects were relatively more impaired in females than males. When comparing *FKRP* c.826C>A homozygous males and females, females showed poorer HRQoL on aspects of bodily pain, social relationships, and emotions, and a higher LGMDR9-related burden on physical aspects and overall burden. Compound heterozygotes reported a higher burden on dysphagia and physical aspects compared to homozygotes but the same overall burden. Level of education was negatively associated with emotional and social aspects of HRQoL but unrelated to overall burden. As expected, level of education was negatively associated with 100% long-term sickness absence, indicating a higher participation in working life. No relationships were detected between living alone and HRQoL but there was a negative trend in all aspects of SF-36 and INQoL, except independence.

Relationships with age or disease burden: In homozygotes, all INQoL subscales correlated with age and/or disease duration except myalgia, emotions, and body image. In compound heterozygotes, none correlations with age or disease duration were detected.

Relationships with symptoms and disease severity: Disease burden was positively correlated with perceived muscle weakness, fatigue, myalgia, walking aids, wheelchair dependency, and PAP therapy, but was unrelated to dysphagia, cardiomyopathy, and sleep quality. Only perceived muscle weakness and fatigue were identified as independent predictors of disease burden.

Prevalence and correlates of fatigue and sleep quality: The prevalence of fatigue was 40%. Fatigue severity was positively correlated with LGMDR9-related myalgia and LGMDR9-related mental distress. The prevalence of subjective sleep disturbance was 49%. Subjective sleep quality was inversely correlated with LGMDR9-related myalgia.

4.4 Paper III

Insomnia – prevalence and relationships with demographic and clinical variables and HRQoL: The prevalence of insomnia was 32%, compared to 20% reported in previous Norwegian general population studies using the BIS [103, 104], and was relatively more frequent among females than males (36% vs 27%). Insomnia was unrelated to wheelchair dependency and PAP therapy. Nevertheless, among respondents with PAP therapy, we noticed that prolonged wakefulness after sleep onset was more frequent and sleep onset latency less frequent compared to those without PAP therapy, whereas early awakening and daytime insomnia symptoms were equally frequent. However, in this case, no statistical testing was performed, since this was not an a priori hypothesis. Insomnia severity was unrelated to age and to physical and social aspects of HRQoL, but showed moderate, negative correlations with mental HRQoL. Insomnia was significantly more common in fatigued compared to non-fatigued respondents (54% vs 21%).

EDS - prevalence and relationship with fatigue: In total, 10% reported EDS. EDS was not significantly more prevalent in fatigued compared to non-fatigued respondents (15% vs 7%).

Unrecognized/untreated SDB: One of 26 PSG candidates had previously recognized but untreated SDB due to lack of tolerance to the PAP therapy. Another candidate met the criteria for hypoventilation among 19 candidates with a successful PtcCO2 monitoring. SA was frequent (4/8 females and 12/18 males) and classified as light in 10/26, moderate in 6/26, and severe in 2/26 candidates.

Relationships between SDB and demographic and clinical variables and HRQoL: All four PSG candidates with a Body Mass Index > 30 and all eight with an EF < 50% had SA. The AHI correlated with advancing age and an EF < 50% but not sex, Body Mass Index, pulmonary function, or oropharyngeal involvement. The AHI did not correlate with the severity of symptoms on the respiratory HRQoL questionnaire. Nevertheless, we observed that the two candidates with severe SA reported most problems. Fatigue severity did not correlate with the nocturnal measurements of sleep and respiration.

Relationships between fatigue and pulmonary function: Among clinical participants without PAP therapy, there was an inverse correlation between fatigue severity and MIP% of a moderate degree (r = -0.46) and with FVC% supine of a weak-to-moderate degree (r = -

0.39). Simultaneously, in the same group, fatigue severity was not correlated with gross motor function (MFM32) or capillary CO2, bicarbonate, or BE. In the subgroup with PAP therapy, who were characterized by a relatively poorer FVC%, a higher age and Body Mass index, and a higher rate of wheelchair dependency, no correlation between fatigue and pulmonary function was found.

A summary of the results and implications of the three papers are provided in Table 4-2.

Table 4-2 Summary of results and implications (epidemiology not included)

	Paper I	**Paper II**	**Paper III**
Main results	Prevalence of PAP: 32%. The majority has restrictiveness. Often combined with SA. **c.826C>A homozygotes:** Bimodal age of onset (childhood onset 75%, adult onset 25%) Risk factors of : -W/C: female, disease duration -PAP: age, poor ambulation -CM: male Half of females were dependent of a W/C by the age of 49 y and PAP at 52 y. Half of males were diagnosed with cardiomyopathy by the age of 55 y. **Other genotypes**: Earlier onset, W/C, and PAP.	During 14 months, muscle weakness and disease burden worsened in c.826C>A homozygotes. All aspects of HRQoL were impaired, except mental health in males and pain. Burden was higher in c.826C>A homozygous females than males and related to fatigue and perceived muscle weakness. Prevalence of fatigue: 40%. FSS correlated with myalgia and mental distress. Prevalence of poor sleep: 49%.	Prevalence of insomnia ~32% in both PAP and non-PAP users, and 53% in fatigued vs. 21% in non-fatigued subjects. Insomnia was associated with fatigue and reduced mental HRQoL. Prevalence of EDS: 10 %. EDS was unrelated to fatigue. Undiagnosed SA was prevalent. The AHI correlated with an EF < 50% and advancing age. Fatigue severity correlated inversely with MIP%, but not with motor function, blood gases, or PSG parameters.
Implications	Increasing attention to respiration/SDB with increasing age and loss of ambulation. Regular cardiac follow-up from diagnosis.	Attention also to mental and social aspect of HRQoL, gender-specific care needs, fatigue, the fatigue-mental distress-myalgia inter-relationship, and sleep issues.	Attention to insomnia in patients with fatigue, impaired mental HRQoL, or PAP, to SA in comorbid CM, and to pulmonary function in fatigue.
Future research	Modifiers in age of onset and CM development. Validation of sex differences.	Interventions on fatigue.	Proper treatment criteria for SDB.

Abbreviations: LGMDR9 = FKRP-related limb-girdle muscular dystrophy R9, HRQoL = health-related quality of life, PAP = Positive Airway Pressure therapy, W/C = wheelchair dependency, CM = cardiomyopathy, FSS = Fatigue Severity Scale, EDS = excessive daytime sleepiness, SA = sleep apnea, AHI = apnea-hypopnea index, EF = left ventricular ejection fraction, MIP% = Maximal Inspiratory Pressure percent predicted, PSG = polysomnography, SDB = sleep-disordered breathing, CBT-I = cognitive behavioral therapy for insomnia, NMD = neuromuscular disorders

5 Discussion

5.1 Discussion of main results

5.1.1 Prevalence

The prevalence of genetically confirmed LGMDR9 in Norway in January 2021 was 2,84/100,000 compared to 1,85/100,000 in January 2009. The diagnostic coverage in January 2021 was expected to be high, as the genetic test had been available in Norway since 2006, and as LGMDR9 had long been known to be a relatively common LGMD in Norway, which increases the diagnostic attention to LGMDR9. Nevertheless, there was a significant delay from age of onset to diagnostic work-up in *FKRP* c.826C>A homozygotes, which may reflect under-recognition in the pediatric population, but also a slowly progressive disorder and unspecific symptoms.

We found that Norway still has the highest prevalence of LGMDR9 and allele frequency of *FKRP* c.826C>A in the general population recorded world-wide. The estimated allele frequency of *FKRP* c.826C>A was only slightly higher than the one reported for Sweden in a genomic database [26], but we found no recorded prevalence of LGMDR9 in Sweden. Furthermore, by reviewing genetic analyses on 127 *FKRP* c.826C>A homozygotes, we replicated previous findings of a 100% association between *FKRP* c.826C>A and the SNP, *FKRP* c.135C>T. This strengthened the theory that *FKRP* c.826C>A is a founder variant and unlikely to arise spontaneously. Since LGMDR9 is closely related to *FKRP* c.826C>A in populations of European origin, and given that this variant is a founder variant, the geographic distribution of LGMDR9 likely results from founder effects of previous European migrations, and genetic drift in small settlements.

The age of the *FKRP* c.826C>A allele is unknown. However, based on the current distribution of LGMDR9, an existing hypothesis is that the *FKRP* c.826C>A allele was spread by the Vikings and general migrations during the Viking Age, hence popularly called the “Viking mutation”. Nevertheless, a founder variant may be thousands of years and, as discussed in Paper I, since the historical waves of migration in Northern Europe have been northward, the variant could have originally been brought to the Scandinavian peninsula by earlier south to north migration followed by accumulation in small, isolated communities.

Prevalence at the county level showed a north-south and a southeast to southwest downward gradient. Particularly, there was a strong gradient between Central Norway and south-west Norway. The systematical pattern and lack of overlapping confidence intervals of the lowest and highest prevalence indicate that random variation is an unlikely explanation. Furthermore, while regional differences in diagnostic coverage could be an explanation, correlations with previously reported genetic substructures [155] support that the difference could be representative. Additionally, there is a well-established national cooperation in the field of NMD in Norway, which makes considerable regional differences in diagnostic coverage less likely.

5.1.2 Diversity and correlations in natural history

The phenotypic differences between *FKRP* c.826C>A homozygotes and the subgroup with other genotypes, mostly *FKRP* c.826C>A compound heterozygotes, align with findings in multiple previous studies (Sect. 1.3). The inter-sibling variation supports that other genetic and/or environmental factors influence the disease evolvement. A large sample of c.826C>A homozygotes allowed assessment of diversity and correlations in natural history without disruptions from genotype effects:

The bimodal distribution in age of onset was a novel finding and suggests modifying factors in the adult-onset subpopulation. This could be further explored in future studies. Wheelchair dependency was positively correlated with disease duration and being a female, and PAP therapy with age and inversely with level of ambulation. The lack of relationship between cardiomyopathy and age, disease duration, and level of ambulation, respectively, aligns with findings in previous studies on LGMDR9 (Sect. 1.3) and suggests that the development of cardiomyopathy depends on other factors than the myopathy. These factors could be genetic or epigenetic. The sex differences were novel findings and raise the suspicion that sex hormones or physical exposure, which tends to differ between males and females, may play a role. Since males were less predisposed to become wheelchair dependent or need PAP therapy compared to females, but more predisposed to develop cardiomyopathy, one may ask whether the latter was caused by higher levels of physical exposure as a result of a relatively preserved motor function and more physical capacity from the nature. The effects of exercise on the heart muscle in LGMDR9 should be further explored.

While sex differences in natural history of muscle disease have been little explored, this study reports statistically significant sex differences in natural history of LGMDR9. The finding of an earlier loss of ambulation in females compared to males corresponds with the previous finding of earlier loss of the ability to run in females with LGMDR9 and more severe muscle involvement on MRI in females with dysferlinopathy, but diverges with studies on other similar diseases (Sect. 1.3). Cardiomyopathy has also been reported to be more common among males than females in Myotonic dystrophy type 1 [156]. Further studies are needed to establish whether the associations with sex observed in the present study are LGMDR9-related. Better physical functioning of males compared to females may be physiological, since testosterone has anabolic effects on skeletal muscle. The findings could also be biased by sex differences in cardiac or respiratory comorbidity (lack of diagnosis-specific biomarkers), healthcare seeking behavior (affecting the detection of cardiomyopathy and/or the initiation of PAP therapy), or response behavior on questionnaires (over or underreporting level of ambulation and age of wheelchair dependency) (Sect. 5.2.3 and 5.2.4).

5.1.3 Temporal relationships

Temporal relationships provide prognostic information to affected people and care providers and guidance for clinical care and trials. Several temporal relationships were studied in the present studies, which brought new insight into various aspects of LGMDR9.

In Paper I, we computed estimated probability curves for endpoints with age as time scale and assessed whether age or duration predicted endpoints (Sect. 5.1.2). In Paper II, we examined correlations between burden and age or disease duration and found that only mental domains were unrelated to both age and disease duration, whereas the other domains showed a worsening trend. We also studied the changes prospectively over 14 months and detected worsening in perceived muscle weakness and overall burden in *FKRP* c.826C>A homozygotes despite a short time frame and a relatively slowly progressive disease. Comparatively, compound heterozygotes showed tendencies towards worsening of weakness and a paradoxical relief of burden. A plausible explanation could be that all the compound heterozygotes included in the prospective analysis were in a wheelchair dependent stage. Most of the meaningful disease progression had thus already taken place, which facilitates the mental, physical, and social adaptation that tends to decrease the burden over time. In Paper III, we found that the AHI was positively correlated with age, which also is the case in the

general population. In contrast, insomnia was not related to age and correlated with mental rather than physical aspects of disease.

5.1.4 Clinical implications

In the absence of causal treatment, symptomatic and supportive treatment is essential. The findings of this research provide fundaments for clinical improvements in several ways.

The results presented in Paper I show that LGMDR9 is physically a debilitating disease and commonly accompanied by cardiomyopathy and/or respiratory failure. Results in Paper II show that both physical, mental, and social aspects of HRQoL are compromised. Lastly, findings in Paper III suggest that insomnia and SA are underrecognized, including insomnia in patients with PAP therapy, potentially affecting the adherence to PAP therapy and thus respiratory outcomes, and including SA in patients with cardiac dysfunction, who are vulnerable. This complexity emphasizes the importance of holistic and interdisciplinary care.

Cardiomyopathy was unrelated to age and disease advancement, whereas PAP therapy was related to advancing age and poor level of ambulation. These findings support the importance of early cardiac monitoring and increasing attention to respiration as the walking function impairs.

Among *FKRP* c.826C>A homozygotes, females reported higher disease burden than males. This could reflect more severe disease in females, as findings in Paper I indicated. However, the perceived burden of disease may be influenced by various stresses or resources in life that also may differ between males and females. Therefore, the findings suggest to increase attention also to gender-specific differences in care needs.

Fatigue was prevalent and an independent predictor of disease burden, suggesting that fatigue needs monitoring and appropriate management. Fatigue was associated with insomnia, and fatigue severity correlated with myalgia and mental distress, which are therefore potential targets for the treatment of fatigue. These relationships align with previous findings (Sect. 1.6.2). Interestingly, fatigue severity also correlated with inspiratory weakness (MIP%) but not blood gases or motor function, suggesting that fatigue may relate to increased work of breathing. This finding advocates for pulmonary assessment in fatigued patients and indicates that MIP may be a meaningful tool in the respiratory follow-up of this population.

The high prevalence of insomnia and the association between insomnia and fatigue and mental HRQoL suggest a need for increased recognition and treatment of insomnia.

According to our analyses, the study participants are not more affected by bodily pain than the general population, and LGMDR9-related myalgia does not impact overall burden of disease. Nevertheless, myalgia did correlate with fatigue severity and negatively with subjective quality of sleep. These are well-known inter-relationships, but it means that myalgia may be relevant also in LGMDR9 as a potential perpetuating factor in fatigue or sleep disturbance.

SA was prevalent and underrecognized, and advancing age and cardiac failure appeared to be risk factors for undiagnosed or untreated SA. While optimal biomarkers of severity and general treatment criteria remain to be established for SA, a European task force has advised to also consider the individual susceptibility in treatment decisions of SA [157]. Consequently, we particularly suggest increased attention to SA in patients with comorbid cardiomyopathy. Furthermore, the fact that AHI did not correlate with meaningful symptoms, advocates for routine sleep studies in vulnerable patients.

5.2 Methodological considerations

5.2.1 Research design

The natural history study (Paper I) included retrospective and cross-sectional analyses. A 2-year follow-up with repetition of standardized measurements was planned but not performed at the time this study was conducted. A longitudinal design with standardized measurements is the gold standard of natural history studies, but is time and resource intensive. A retrospective design also allows temporal analyses and saves time and resources. Nevertheless, retrospective studies rely on the data available, which may be incomplete and non-standardized, as was the case with the medical records. On the positive side, medical record is a source of reliable and life-long clinical data. The project-specific questionnaire and the neurological clinical examinations in the clinical part of the study were used to compensate for the missing data of the medical records and for additional cross-sectional analyses. The cross-sectional analyses provided an overview of the study population, such as demographic data and proportions of participants with wheelchair dependency, PAP-therapy, and cardiomyopathy, but provides little information about the course of disease.

The HRQoL study (Paper 2) included prospective and cross-sectional analysis. A prospective design with repeated measurements was chosen to study the temporal changes of HRQoL and the measurement variability. Three time points of measurement were chosen to allow prospective analyses of change, measurement variability, and correlation analyses while adjusting for measurement variability and data dependencies via linear mixed model regression. This was considered to provide a higher level of evidence in the study of correlations with HRQoL compared to a cross-sectional correlation study using only one data point per variable per participant. This design also increased the overall participation rate.

The sleep study (Paper III) included only cross-sectional analyses, providing the lowest level of evidence of causality. To establish causality, an interventional design is required. Nevertheless, the intention was to explore the prevalence of sleep disorders and correlations with sleep and fatigue, and a cross-sectional study is a feasible design to detect relationships. Significant relationships can then give rise to more targeted studies designed for providing evidence of causality. In correlations, the directions and underlying mechanisms cannot be established, including between fatigue and myalgia/mental distress/perceived burden of disease/ insomnia/MIP%, between perceived muscle weakness and perceived burden of disease, between insomnia and myalgia/mental distress, and between the AHI and cardiac failure, respectively. Consequently, it can only be concluded that e.g., fatigue management *may* be a strategy to reduce burden and that myalgia, mental distress, and insomnia are *potential* targets for the treatment of fatigue, moreover, that cardiac failure increases the probability of also having SA. Some relationships may be bi-directional, e.g., fatigue-mental distress, whereas other relationships cannot be bi-directional, e.g., advancing age can not be a consequence of an increased AHI. Relationships may also be confounded or mediated by other variables (Sect. 3.7 and 5.2.3).

5.2.2 Measures

The study included validated self-report measures and standardized objective measurements. Strengths and weaknesses of these measures are discussed in the current section.

Self-report measures

The use of generic instruments (FSS, PSQI, BIS, ESS, and SF-36) made it possible to compare with previous findings in general populations. There were some particular

considerations related to the BIS. In the absence of biomarkers for insomnia, chronic insomnia disorder is entirely a subjective diagnosis. Consequently, self-report instruments can be used for prevalence estimations. Nevertheless, the BIS can under or overestimate prevalence of chronic insomnia disorder. While the BIS is based on ICSD-3 criteria, BIS does not cover all types of daytime impairment in ICSD-3, such as cognitive impairment, reduced motivation, or negative mood. Furthermore, the BIS does not rule out the ICSD-3 exclusion criteria: when symptoms are related to sleep environment, restricted allocated time to sleep, or when the insomnia symptoms are better explained by another condition. The latter would require a clinical interview and examination and in selected cases additional investigations to rule out differential diagnoses. In patients with PAP therapy where poor adjustments cause the insomnia symptoms, chronic insomnia disorder would be an inappropriate diagnosis. Another source of inaccurate prevalence estimation is that the BIS asks for *the number of days per week in the last three months* the specific symptom was experienced. An average of ≥ 3 days per week the last 3 months does not necessarily equal 3 months duration with ≥ 3 days per week, which is an ICSD-3 criterion. Additionally, 3 months is a long recall period, which opens for recall errors and recall bias (Sect. 5.2.3). The original version, which was the one that was validated, had a recall period of one month according to the previous diagnostic criteria (Sect. 3.3.2).

INQoL was the only NMD-specific measure applied. While INQoL and SF-36 both contain physical, social, and mental aspects of HRQoL, INQoL aims to selectively measure how the NMD affects these areas. Nevertheless, it is debatable whether it is possible to separate satisfaction on different aspects with regard to the disease from general satisfaction on the respective aspects, and consequently whether the burden only reflects the burden of LGMDR9. An important strength of INQoL is that it measures the impact and overall impact on HRQoL (i.e., disease burden) in addition to perceived health status. It is well known that the levels of disability and impact are not proportional, and that mental adaptations and life adjustments tend to reduce the burden over time, a phenomenon named the disability paradox [158]. Validations of instruments are always limited, e.g., with regard to the sample, properties tested, methods, and time and place. INQoL was validated on a sample of heterogeneous muscle diseases and prior to the COVID-19 pandemic, and was not adequately validated on sensitivity. This made interpretation of the detected changes difficult. Nonetheless, the findings of significant changes within a relatively short time frame and high

or moderate rank-order stability of the subscales suggest suitability for monitoring in comparative trials.

Objective measurements

Body Mass Index is a widely used measure in medicine and feasible as it is easy to measure in a standardized way and has the same healthy values for males and females.

Echocardiography and pulmonary function tests were performed with standardized protocols. Nevertheless, pulmonary function tests also depend on the effort of the participants and their ability to perform the test adequately. Those with significant diaphragm weakness may not be able of performing the supine spirometry. Others may have air leakage.

The PSG study was uncontrolled since the aim was to study correlations and the occurrence of unrecognized SDB based on diagnostic criteria rather than comparing with healthy individuals. The PSG was conducted the second night, which reduced the first night effects of a new setting and sleeping environment. Moreover, PSG was installed at 3 p.m., which gave the participant time to adapt to the equipment. Serial night PSG would be theoretically preferable, but resource-intensive, burdensome for the participants, and possibly affect their performance on other tests. It would also probably lead to drop-offs after the first night. Physical tests were spread over two days. This minimized exhaustion and myalgia, which may affect sleep. However, some patients did report that they had napped during the day which they normally did not do, woke up with unnormal pain, or slept poorer than normal. One reported better sleep than normal. However, the quantity of total and deep sleep and the sleep efficiency indicated successful registrations.

The diagnostic criteria of SDB may not represent optimal treatment criteria, since diagnostic criteria are mostly based on expert opinions rather than comparative trials. For example, although the AHI is associated with a multitude of poor health outcomes and is thus a relevant biomarker, it has several flaws as a biomarker of severity. The traditional subdivision of SA into light, moderate, and severe by the AHI is arbitrary [159]. The AHI does not reflect the type of events (such as arousal versus desaturation) or the intensity of the events (such as duration and depth of the desaturations). Furthermore, the AHI is influenced by the measuring equipment, the scoring rules applied, and sleeping position and sleep architecture during registration [159, 160], which may vary from night to night and be influenced by the test itself

("the Heisenberg effect") [161]. Lastly, the AHI correlates poorly with daytime symptoms, and its negative clinical impact may also depend on comorbidity [157].

The absence of correlation between the single-night objective sleep measurements and the habitual symptoms, measured by FSS and the sleep-related questionnaire, could potentially be caused by poor metrics or poor representativeness of the sleep registration. Additionally, since PSG reading is subject to inter-rater variance, the division between two raters may potentially have deflated the correlations.

Although transcutaneous CO2 monitoring has been acknowledged in the diagnosis [117] and treatment of chronic hypoventilation [162, 163], the accuracy compared to invasive arterial blood gas is limited [164]. This raises concerns about the sensitivity and reliability. We assessed correlations between mean nocturnal PtcPCO2 and morning capillary blood gas, which were moderate to strong (PCO2: r = 0.59, bicarbonate: r = 0.69, and base excess: r = 0.67). Nevertheless, the measurements may still be inaccurate. Additionally, there were more technical issues than expected. For various reasons, the automatic drift-corrected PtcCO2-values were missing in several cases. These measurements were excluded, which reduced the completeness of data.

5.2.3 Bias

Bias are systematic errors that lead to over or underestimation. The present PhD project was exposed to bias in the recruitment, data collection, and analyses.

Selection bias: All identified subjects with LGMDR9 were invited to the cohort study, and the coverage should be near 100% with the methods used. Therefore, the invitation process in the cohort study, and thus natural history study, was not biased. However, the cohort may be skewed by nonresponse (self-selection) bias. A high participation rate, a comparable number of males and females, and the age-, genotype-, and geographic distribution in the cohort were indicators of a representative sample. Participation in the cohort study required that one had received and read the letter, consented to the collection of extracts from patient records, completed the questionnaire, and ultimately posting the respond letter. Non-participants may be people with changed address or that do not read all mail, people with poor reading or writing skills, severe physical, mental, or cognitive comorbidity, busy lives, fear of sharing personal information, or people with low motivation due to advanced disease, minimal

symptoms, or a desire not to focus on the disease. People who are likely to participate are those with a personal agenda, such as obtaining additional tests or a hope for a cure in the future, and people with an academic interest in research or a sense of duty. Psychosocial issues may either be a reason to participate (achieve better services) or not participate (e.g., due to lack of motivation or not wanting to share personal information). Since participation did not require any travel or physical examination (voluntary), it is reasonable to believe that there is no significant nonresponse bias in the cohort regarding physical, mental, or social health areas. Reminders were sent to reduce nonresponse bias.

The respondents of the HRQoL and sleep surveys were recruited from the cohort, which we believed was representable in all health areas. The postal administration of questionnaires prevented exclusion of persons who do not use the internet. The high response rates achieved lower the risk of nonresponse bias. Expectedly, any selection bias would primarily skew the absolute measurements, including the HRQoL scores and prevalence of poor sleep, insomnia, EDS, and fatigue, and presumably in the poorer direction considering personal agenda/motivation. Subgroup comparisons of HRQoL and correlation analyses with HRQoL and sleep are likely to be more robust to nonresponse bias. The HRQoL scores were not interpreted in this study. To evaluate the status of HRQoL, we rather used comparisons with reference data, and it can be expected that similar nonresponse bias exists in the reference data as in the study population.

We expected the clinical sample to have a relatively higher level of physical functioning, since clinical participation required a 2-day stay at the hospital, and for most of the participants a return flight. Nevertheless, the clinical sample was comparable to the cohort with respect to age, sex, wheelchair dependency, and PAP therapy and comparable to the postal survey respondents with respect to fatigue prevalence. The rate of PSG candidates was high among eligible clinical participants, but relatively lower in females. Unfortunately, the reasons for not undergoing PSG (not offered or not wanting and why not wanting) were not registered. One plausible reason for not wanting to undergo PSG is wheelchair dependency due to the practical inconvenience, i.e., a self-selection bias. Correspondingly, there were only two wheelchair dependent PSG candidates. Since the rate of wheelchair dependency was relatively higher in female clinical participants, that could possibly explain why the participation rate was lower in females. A higher rate of wheelchair dependent participants in the PSG/PtcCO2 study could potentially have uncovered a higher rate of SDB, especially

hypoventilation, but it would presumably not affect the correlation analyses. The selection criterion for the PSG study (i.e., not using PAP therapy) contributed to the predominance of males among PSG candidates, since relatively more females had PAP therapy. The sex ratio influences on the prevalence of SA, because SA is more common among males in in the general population. The gender imbalance was handled by presenting the results by sex.

Observer bias: A type of cognitive bias that arises when the judgement in the data collection is systematically affected by the expectations. This may occur when the observer is not blinded to the subject´s group affiliation (e.g., genotype or sex) and the research hypotheses. In this PhD project, both the PhD candidate and several co-authors were involved in parts of the data collection. Optimal for sake of the project would be to use external observers, but was not feasible.

Missing data: Variable routine follow-up and reporting in patient records limited data completeness. To compensate for missing clinical neurological data, it was complemented with data from the clinical part of the study and the study-specific questionnaire.

Biased questions: Arises when the responses are influenced by the framing of the items, e.g., leading or loaded questions, double-barreled items, and polarity. This issue was minimized by using validated questionnaires.

Response bias: Arises due to various reporting behavior on self-report questionnaires. Some may underreport problems (e.g., due to shame), while other may overreport (e.g., due to dissatisfaction with the services). The fact that females reported poorer HRQoL on INQoL, may potentially be due to males underreporting issues or because they perceive the questions differently. By using the SF-36 to compare with normative data, we could check whether the sex differences corresponded with the sex differences in the reference population, which they did. Recall bias occurs when recall errors skew the results in either direction and can be prevented by using questionnaires with short recall periods. The 3-month recall period of the BIS was relatively long compared to e.g., the SF-36 with 1 month and INQol with zero recall period. There is a risk that the responses rather reflect the last couple of weeks, and hence, that the BIS overestimates the prevalence of chronic insomnia.

Common method bias (CMB): Occurs when the relationship between the independent and dependent variable is affected by common measurement methods [165]. This typically applies

to cross-sectional self-report surveys where multiple variables are measured at the same time point and with the same source (a questionnaire). Similar formate of the items or item scales tend to generate similar responses, and individual item responses are influenced by the order of the items (cognitive carry-over effects). To prevent CMB, the instruments administered together were not put in a specific order. The risk of CMB was also reduced by the variation in format and item scales between and sometimes within the measures. Furthermore, the sleep survey and the HRQoL survey used for correlation analyses between HRQoL and sleep were separated by 1 month.

Confounding bias: Occurs when a third variable affects both the predictor and the outcome variable so that the estimate of the association between the predictor and outcome variable is significantly affected. This was handled by multiple regression.

5.2.4 Statistical considerations

The *Hardy-Weinberg equation* was used to estimate the carrier frequencies at national level, although regional differences in prevalence and clustering within the counties indicate deviation from the equilibrium. Nevertheless, the equation has proved to perform well as an approximation even when the assumptions of equilibrium are not met [151].

The Wilson score interval for estimation of random variation in prevalence relies on binomial probability, meaning that the probability of LGMDR9 is the same for all individuals within the defined region. In reality, there are subpopulations (e.g., geographic or ethnic clusters) with a different carrier frequency. Such dependencies are not accounted for in the estimation of Wilson score intervals.

Some sample sizes were low. This decreases the power, i.e., increases the chance of false negative tests (Type 2 errors). Correction for multiple testing would have increased this risk. Simultaneously, by not correcting for multiple testing, the risk of false positive tests (Type 1 errrors) increases. However, since the present studies were exploratory and not a clinical trial, false positive test would not have any foreseeable negative clinical consequences, whereas false negative tests would limit the utility of the study. Correction for multiple testing was performed in Paper I after request from one of the referees. However, there is no consensus about how such corrections should be performed, which reduces the utility. Furthermore,

considering the methodical limitations novel findings need replication and validation either they remain significant or not post correction for multiple testing.

The survival analyses concerning cardiomyopathy and PAP therapy are prone to survival bias, because the time of recognition of cardiomyopathy or need for PAP therapy depends on the follow-up. A late first cardiac or pulmonary examination and long follow-up intervals tend to prolong "survival" and thus underestimate the cumulative probability. Regarding ambulation, the survival curve relied mostly on self-reports and was therefore prone to response bias and recall errors.

The statistical analyses were selected and performed by the author of this thesis (the PhD candidate). Consequently, there exists a risk of confirmation bias, i.e. a cognitive bias where results that fit an a priori hypothesis are favored.

5.2.5 External validity and generalizability

The external validity of the observational studies was strengthened by high participation rates, high data completeness on the self-report questionnaires, and by a clinical sample that was representative of the cohort. The PSG study was limited due to a low sample size. It was considered to provide sufficient evidence that SA is underrecognized in the target population, but may not reflect the extent of unrecognized SDB as a whole.

This PhD project was a nation-wide study of LGMDR9 in Norway. Nationally, certain findings concerning HRQoL and sleep may apply to other NMD populations due to common disease characteristics and professional community. In a global setting, the generalizability may depend on differences in genotype distribution, clinical practice, health care services, and culture. Multiple outcomes of the present studies depend on clinical practice, such as the recognition of cardiomyopathy and SDB and initiation of PAP therapy. Disease burden may depend on political system, public health services, and culture. Furthermore, the results of the subjective measurements (HRQoL, fatigue, and sleep) may not be generalizable in time. Although the surveys were conducted in relatively normal COVID-19 free periods, the pandemic may have influenced on the results. Nevertheless, comparable cross-sectional measurements of HRQoL and absent prospective change in the aspects of social relationships, myalgia, fatigue, and emotions suggest that pandemic effects were minor.

5.2.6 Conclusion and future perspectives

This work extends knowledge of epidemiology and natural history of LGMDR9 and brings new insight into HRQoL and sleep disorders in people with LGMDR9.

The epidemiological study gives an update of prevalence and genetic diversity of LGMDR9 in Norway and documents regional differences. The study also strengthens evidence that *FKRP* c.826C>A is a founder variant and unlikely to occur spontaneously, since it has so far shown 100% association with *FKRP* c.135C>T (SNP).

The natural history study corroborates previous findings of characteristics and diversity. Moreover, through a large sample and original analyses it provides novel findings associated with the *FKRP* [c.826C>A/c.826C>A] genotype. The bimodal distribution in age of onset may reflect modifiers in adult-onset disease, which could be further explored. Time-to-endpoint and regression analyses provide prognostic information and show significant sex differences. Further studies are required to conclude whether the sex differences are LGMDR9-related or biased, and to identify possible mechanisms. The paradoxical finding of more retained physical functioning and increased disposition to cardiomyopathy in males compared to females is surprising. In particular, it raises questions about the impact of sex hormones and exercise on skeletal muscle and heart muscle in LGMDR9, and importantly, whether exercise can have negative impact on the cardiac muscle given the assumption that males had been more exposed. Additionally, cardiomyopathy development appears to be unrelated to disease advancement, which supports that other genetic or epigenetic factors are involved. This should be subject of further research. PAP therapy correlated with advancing age and poor walking function. According to our analyses, the latter explained why PAP therapy was more frequent in females. With regard to clinical follow-up, these findings support the importance of early cardiac monitoring and increasing attention to respiration as the walking function impairs. Finally, there was a considerable delay from onset to diagnostic work-up. Increased attention to signs and symptoms of muscle disease on health checks of children may contribute to early diagnosis, which is key to timely interventions such as cardioactive medication and, in the future, disease-modifying therapy.

This research increases understanding of the subjective nature of LGMDR9 and how it affects people´s live. Importantly, it has identified potential ways to improve HRQoL in this population. The findings suggest increased attention also to mental and social aspects of

health and to gender-specific differences in care needs. Besides muscle weakness, fatigue proved particular important to the patients. The high prevalence and potential reversibility of fatigue advocates for fatigue monitoring and management. Correlations suggest particular attention to myalgia, mental distress, insomnia, and respiratory muscle weakness in fatigued patients. Whereas previous studies indicate that CBT and exercise therapy alleviate fatigue in some NMD, effective and appropriate treatment in LGMDR9 remains to be established.

New insights into sleep disorders in LGMDR9 were obtained. Insomnia correlated negatively with mental HRQoL and was prevalent, including among patients with PAP therapy, where it can potentially also affect the compliance to PAP therapy and thus even respiratory outcomes. We suggest increased recognition and treatment of insomnia in the interdisciplinary care of this population. SA was underrecognized among the sleep candidates, including those with cardiac failure, who are vulnerable. The regression analysis of the AHI indicates that an impaired EF should be considered a risk factor of SA. Furthermore, the lack of correlations between the AHI and meaningful sleep-related symptoms, advocates for routine sleep studies in vulnerable patients. The lack of respiratory evidence-based guidelines for LGMD is a barrier in the diagnosis and treatment of SDB and therefore an area for further research.

Several results are useful information to interventional studies. Clinical trials should be aware of potential sex differences in natural history. Fatigue appears to be a meaningful patient-reported outcome variable given its close relationship with overall burden. Moreover, MIP% is potentially a meaningful outcome measure due to its correlation with fatigue severity, and INQoL demonstrated promising monitoring properties.

Main limitations of this work are the reliance on self-reported data and clinical practice, the potential influences from the COVID-19 pandemic, and the increased risk of false positive findings owing to multiple testing and common method bias. Resultantly, novel observations should be replicated and validated in future studies. It should also be kept in mind that correlations in observational data do not imply causation, and that even multiple regression is prone to bias in establishing causal relationships.

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Paper I

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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)

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Gender-related LGMDR9 differences

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

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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.

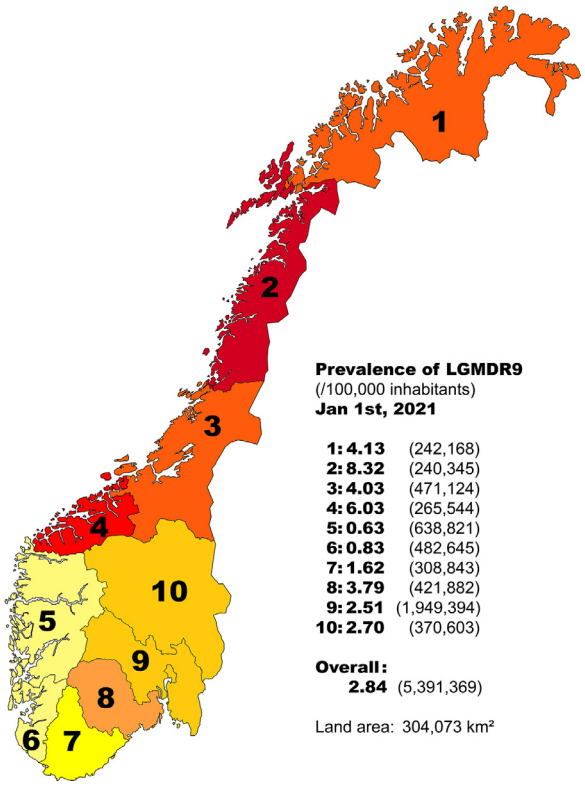

Fig. 1. Map of Norway showing minimum LGMDR9 prevalences and population sizes on national and county level as of 1st of January 2021. Oslo was merged with Viken county due to the small area and similar prevalence (number 9). Colour intensity correlates with the whole number of cases with LGMDR9 per 100,000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

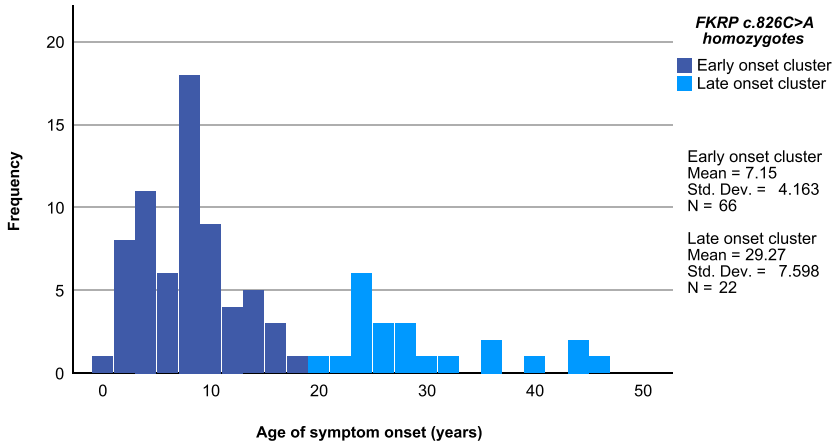

Fig. 2. Histogram demonstrating the distribution of age at onset in the FKRP c.826C>A homozygous participants (n = 88). Subjects are divided in an early and late onset group according to a two-step cluster analysis.

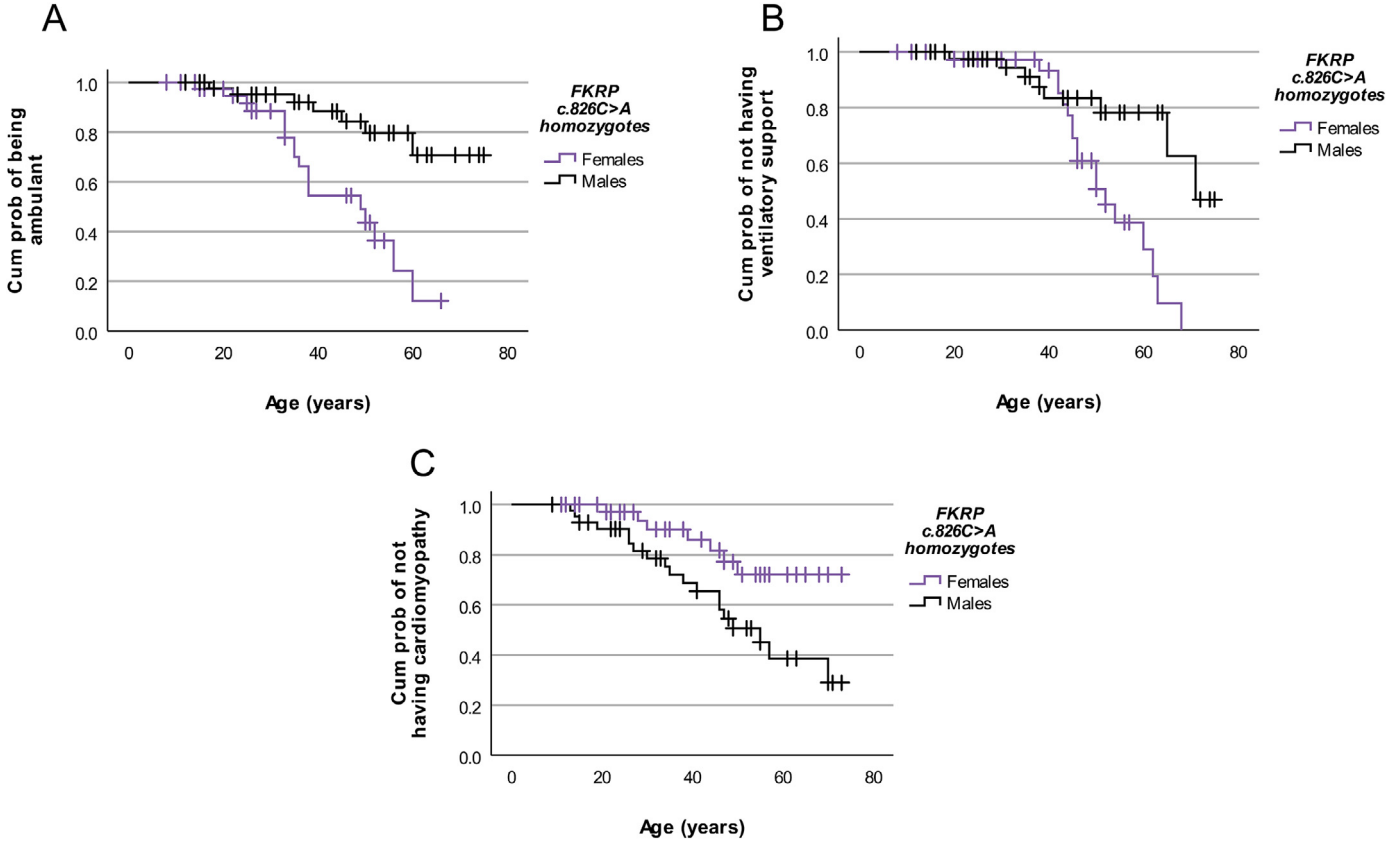

Fig. 3. Kaplan-Meier curves showing cumulative probability with age of (A) being ambulant, (B) not having initiated ventilatory support, and (C) not having cardiomyopathy, respectively, in FKRP c.826C>A homozygous males (n = 45) versus females (n = 43).

(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

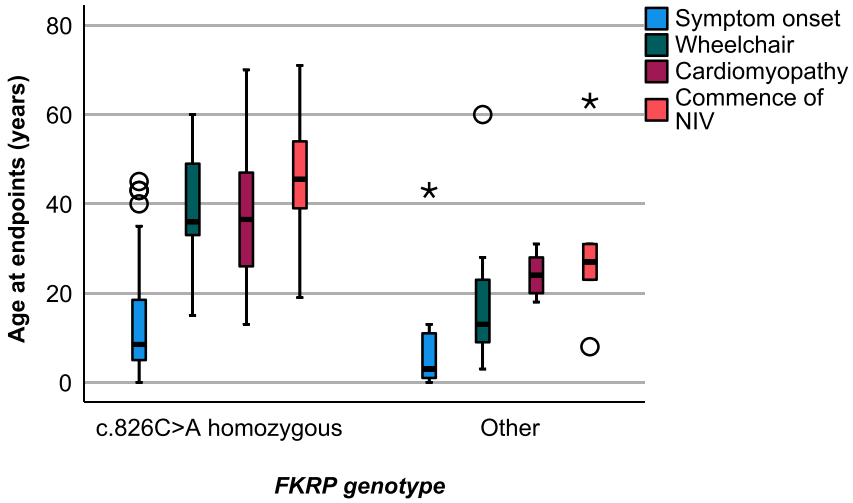

Fig. 4. Box plot showing age at symptom onset, wheelchair dependency, cardiomyopathy and initiation of non-invasive ventilatory support (NIV) in subjects with FKRP c.826C>A homozygous genotype (n = 88) versus other FKRP genotypes (n = 13).

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

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Appendices

Fig. A1

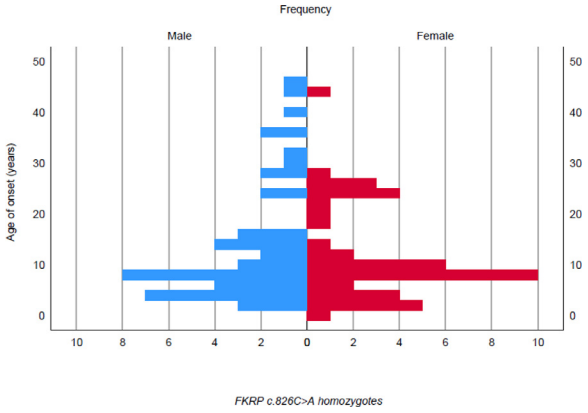

Fig. A1. Histogram demonstrating distribution of age at onset in the FKRP c.826C>A homozygous participants divided into genders. (For interpretation of colour please refer to online publication.)

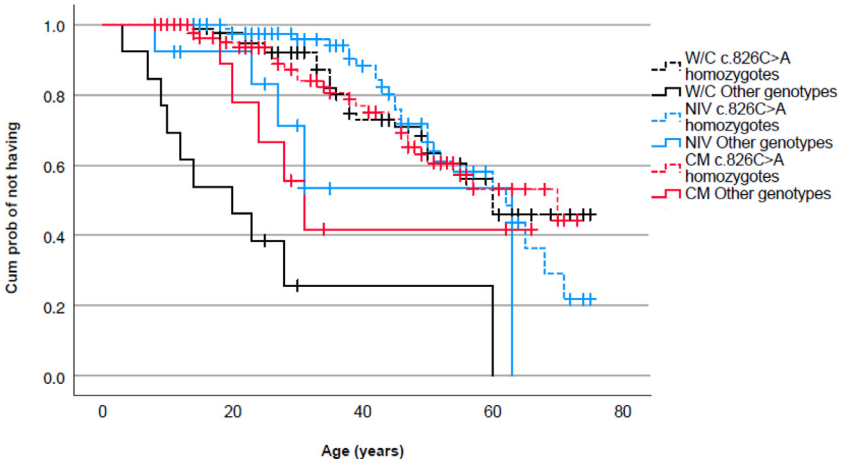

Fig. A2. Kaplan-Meier curves showing cumulative probability with age of not being wheelchair dependent, initiated non-invasive ventilatory support or developed cardiomyopathy, respectively, in FKRP c.826C>A homozygotes versus the cohort of other FKRP genotypes. (For interpretation of colour please refer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

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Research Report

Health-Related Quality of Life in FKRP-Related Limb-Girdle Muscular Dystrophy R9

The Norwegian LGMDR9 cohort study (2020)

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Abstract.

Background: Limb-girdle muscular dystrophy R9 (LGMDR9) is a chronic progressive hereditary muscle disease, related to the Fukutin Related Protein (FKRP) gene, that may cause major disabilities, cardiomyopathy, and ventilatory failure. Knowledge of how LGMDR9 affects health-related quality of life (HRQoL) is relevant in treatment and care.

Objective: To investigate HRQoL in the Norwegian LGMDR9 population over 14 months and relation to fatigue and sleep quality.

Methods: Participants (16+ years) of the Norwegian LGMDR9 cohort study completed two HRQoL measures, i.e., Individualized Neuromuscular Quality of Life questionnaire (INQoL) and the 36-item Short Form (SF-36) at baseline, 8, and 14 months and measures of fatigue and sleep quality at 9 months.

Results: HRQoL response rate was 84/90 (75 c.826 C > A homozygotes and nine c.826 C > A compound heterozygotes). Compared to Norwegian normative data, all SF-36 domain scores were impaired ($p \leq 0.006$) except mental health in males ($p = 0.05$) and pain scores. During 14 months, perceived muscle weakness and the INQoL index (disease burden) worsened in c.826 C > A homozygotes. Compound heterozygotes reported more dysphagia and physical difficulties than homozygotes and showed a tendency towards worsening in weakness over time but some improvement on the INQoL index. Homozygous females reported generally poorer HRQoL and a higher burden than males. The INQoL index was related to perceived muscle weakness and fatigue, and fatigue to myalgia and mental distress. The prevalence of fatigue and poor sleep was 40% and 49%, respectively.

Conclusions: The 14-month follow-up period shows a worsening of perceived weakness and burden in c.826 C > A homozygotes, which can then be expected. The prevalence and impact of fatigue indicate a need for awareness and treatment of fatigue. Myalgia and mental distress are potential targets in the treatment of fatigue, which future studies need to establish. Sleep issues and gender-specific care needs also require attention in LGMDR9.

Keywords: Muscular dystrophies, Limb-girdle, quality of life, sleep quality, fatigue

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INTRODUCTION

Limb-girdle muscular dystrophy type R9 (LGMDR9) is a rare autosomal recessive disease caused by pathogenic variants in the Fukutin Related Protein gene (*FKRP*) [1]. It is more frequent in Northern European populations, with the highest prevalence recorded in Norway; 2.84/100,000 [2]. In European populations, *FKRP* c.826 C > A is the most common disease-causing variant, and c.826 C > A homozygotes tend to have a milder disease progression than c.826 C > A compound heterozygotes [3–5]. The disease typically presents with slowly progressive proximal lower limb weakness at a young age and commonly leads to wheelchair dependency, cardiomyopathy, and eventually ventilatory failure [1, 2, 5].

While no disease-modifying or curative therapy is currently available for LGMDR9, affected individuals are provided supportive care. This may include symptom-relieving medications, cardiorespiratory-, physical-, occupational, or cognitive-behavioral therapy, providing access to user-controlled personal assistance, and efforts to facilitate social participation [6, 7]. Patient Reported Outcome Measures (PROM), such as Health-Related Quality of Life (HRQoL), are important tools to understand the patients´ experiences and provide the necessary supportive care. Despite some conceptual and methodological challenges [8], HRQoL is considered important endpoints in clinical research and health planning [8, 9] and increasingly emphasized as evidence of utility or treatment success. HRQoL measures encompass both generic and disease-specific versions. Generic HRQoL enable comparisons between heterogeneous diagnostic groups and between patients and general populations and may be used to calculate quality-adjusted life years for cost-utility analyses. Disease-specific measures target disease-related symptoms, effects, and burdens and may be more relevant in clinical research aimed at monitoring disease progression or treatment-related benefits. Most HRQoL instruments measure self-perceived health status in physical, mental, and social aspects of health, whereas some also include personal evaluations by the respondents [10] to better reflect the «true» level of HRQoL.

A systematic literature review reports strong evidence for disease severity, fatigue, pain, and mood as important predictors of HRQoL in muscle diseases, moderate evidence for the role of female gender, advancing age, and poor sleep, and weak evidence for disease duration and employment [11]. A recent study indicated that LGMDR9 is associated with considerable symptoms of pain [12]. We have found no study reporting HRQoL in LGMDR9 patients. In all, only a handful of qualitative [6, 7] and quantitative studies [13, 14] have been published on how LGMD impacts daily life.

In the present study, we investigated the magnitude of changes in HRQoL in a Norwegian LGMDR9 cohort during a 14-month period and explored health areas with potential for improvement, subgroups at heightened risk for poor HRQoL, and symptoms of particular importance to patients. Additionally, we investigated the prevalence and correlates of fatigue and poor sleep quality.

MATERIALS AND METHODS

Participants

The present study is a part of "The Norwegian LGMDR9 cohort study" at the National Neuromuscular Centre Norway (NMK), University Hospital of North Norway (UNN). A total of 153 subjects (135 adults, i.e., 16+ years) in Norway with clinically and genetically confirmed LGMDR9 were invited to the cohort study. Of these, 101 subjects gave consent and participated [2]. The participants completed a study-specific questionnaire, and patient notes were collected from the specialist health care. All adults (16+ years) in the cohort (90/101) were invited to the present study of HRQoL.

Instruments

The 36-Item Short-Form Health Survey (SF-36) version 1

The Norwegian version of SF-36 v. 1 [15] is a generic profile-based HRQoL measure that yields nine 0-100-point subscales (from lowest to highest HRQoL): physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotions, mental health, and change in health over the past year. As in the SF-36 algorithm [16], missing values were replaced with the subjects' mean score for the completed items on the respective scale given a minimum of 50% subscale completion. SF-36 is extensively validated [17, 18] and widely used including on patient populations with muscle diseases [11].

The Individualized Neuromuscular Quality of Life queastionnaire (INQoL) version 2.0

INQoL [19] v. 2.0 is a disease specific measure comprising seven symptom domains (i.e., muscle weakness, myalgia, fatigue, myotonia, diplopia, ptosis, and dysphagia), five functional or "life domains" (i.e., activities related to daily living/leisure/work, independence, social relationships, emotions, and body image), and two treatment domains (i.e., perceived and expected treatment effects). The myotonia, diplopia, ptosis, and treatment domains were not included in this study as they were considered irrelevant. Myotonia, diplopia, and ptosis are not reported in LGMDR9. Each domain contains items of severity, impact degree, and impact importance. The item scales are 7-point Likert type and categorical combined as each number also has a categorical description, from either nothing or very little, to extreme. Raw scores are transformed into domain scores ranging between 0 (no burden) and 100 (maximal burden) according to a weighted scoring algorithm. Additionally, the items on impact and impact importance of each life domain are aggregated into one sum score - the INQoL index - as a proxy of disease burden or total impact on HRQoL. Missing data were replaced according to the scoring algorithm.

INQoL is the first HRQoL instrument developed for neuromuscular disorders and has been translated to multiple languages [20]. The Norwegian version is the result of a linguistic validation methodology with forward-backward translation and tested on 100 outpatients with 70 respondents at NMK with various neuromuscular diseases proving satisfactory internal validity as well as concurrent validity with the SF-36 (unpublished data).

The Pittsburgh Sleep Quality Index (PSQI)

PSQI assesses self-reported sleep quality [21]. The Norwegian version [22] was used. PSQI has proven good reliability and validity, it is widely used in screening for sleep disturbances in both clinical and non-clinical populations [23], and has been applied in populations with other types of muscular dystrophies [24, 25]. Based on the items, seven component scores (range 0-no to 3-severe difficulty) are created and summed to one global score (range 0–21) with a cut-off score of >5 indicating significant sleep disturbance. Missing data were not imputed.

The Fatigue Severity Scale (FSS)

FSS [26] indexes the level of fatigue. It is a generic psychometric measure validated across multiple populations including a Norwegian general population [27]. It consists of nine items rated on a 7-point Likert scale, and the FSS score is calculated as the average of the item scores [26]. The revised cut-off score of ≥ 5 (from ≥ 4) was recommended to indicate severe fatigue in a Norwegian FSS validation study [27]. Missing data were not imputed.

Procedure

The HRQoL instruments were distributed in paper versions by regular mail to adult participants of «The Norwegian LGMDR9 cohort study» at three consecutive time points: 2020-June, 2021-February, and 2021-August, respectively. One reminder was sent per administration. In addition, FSS and PSQI were administered one month after the second HRQoL collection. The second HRQoL collection was used for correlation analyses between HRQoL, FSS, and PSQI. Baseline HRQoL was used for correlation analyses between INQoL and SF-36 and for comparison with reference data and of subgroups except genotype subgroups (c.826 C > A homozygotes vs c.826 C > A compound heterozygotes). To enable inclusion of all compound heterozygotes, who were few, the first responses of each unique participant was used rather than the baseline HRQoL. Repeated measurements of HRQoL were applied to evaluate the level of fluctuations in cross-sectional measurements over time and to measure longitudinal changes at the individual level and the rank-order stability of the subscales (Sect. "Statistical analyses"). Social and clinical background data were collected from the questionnaire of the main study and included marital status, living situation, work status, educational achievement, activities of daily living (ADL), ambulation, use of Positive Airway Pressure (PAP) therapy (i.e., mask-based therapy for e.g., sleep apnea or hypoventilation), cardiac involvement, Percutaneous Endoscopic Gastrostomy (PEG), and consumption of pain medication for myalgia. Higher education was defined as at least one year at university or college. Age at disease onset, for calculation of disease duration, was primarily collected from patient notes. Disease onset was defined as the first clinical sign or symptom of muscle disease, such as muscle weakness, myalgia, or myoglobinuria and did not include incidental finding of elevated muscle enzymes. Norwegian normative SF-36 data, used as reference, were

obtained from published material [28, 29], and social population statistics from Statistics Norway [30].

Statistical analyses

Data were analyzed using IBM SPSS Statistics for Windows (Version 27.0. Armonk, NY: IBM Corp.). Distribution of continuous variables are described using mean and standard deviation (±SD), or median and interquartile range (IQR), as appropriate. For simple group comparisons, independent *t*-test with bootstrapping or Kruskal-Wallis (multiple subgroups) was applied. Bootstrapping was always performed with 5000 resamples. In comparisons with normative SF-36 data, independent *t*-test was found acceptable given the sample sizes. In genotype comparison, multiple linear regression (MLR) with bootstrapping was applied to adjust for sex and age. To assess the change in INQoL domains from baseline to 14 months, paired *t*-test with bootstrapping (homozygotes), or paired Wilcoxon-signed rank test (compound heterozygotes due to a very limited sample size) was used. Categorical variables are presented as frequencies. Pearson chi-square was used for variable cell comparisons, and effect size is presented as odds ratio (OR) with confidence interval (CI). Bivariate correlations were examined with Pearson correlation (r) on normal distributions, otherwise with Spearman rank correlation (r_s). Within the context of the current study, we considered correlation coefficients < 0.40 as weak, 0.40–0.69 as moderate, ≥ 0.70 as strong, and ≥ 0.90 as very strong.

For repeated measurements, we used generalized linear mixed models (GLMM) as it is a flexible regression analysis with regarding handling of missing data and data dependencies. The rank-order stability of the HRQoL domain scores was estimated as an Intraclass Correlation Coefficient (ICC) as the ratio between the intersubject variance (random intercept) and the total variance in a model without any fixed effects. ICC values < 0.50, 0.50 – 0.75, 0.75 – 0.90, > 0.90 may be considered as poor, moderate, good, or a high degree of rank-order stability, respectively. A high ICC thus means that if changes in HRQoL occur, the participants show the same change pattern with low variability in change across time. In order to regress the independent variables on the repeatedly measured dependent continuous variables of interest (perceived muscle weakness and the INQoL index), GLMM regression was applied. Choice of covariance structure for the residual matrix was decided based upon which structure that yielded the lowest Bayesian Information Criterion value. All GLMM analyses were performed with robust estimation of the standard errors. To identify predictors of dependent continuous variables measured once (FSS and PSQI), MLR with backward elimination was applied. The selection of independent variables in GLMM, or MLR before backward elimination, was based on the *p*-values of simple regression analyses of the variable of interest ($p < 0.20$ considered relevant). Age and sex were included as control variables. Assumptions of normality of the MLR residuals were checked using Q-Q plots, skewness, and kurtosis, homoscedasticity by scatter plotting predicted versus residual values, and influential cases by Cook´s distance. The alpha level was set to $p < 0.05$. Considering the exploratory nature of the study and small subgroups (low power), correction for multiple testing was not used in this study [31].

Approvals and patient consent

All participants provided a written informed consent for the collection and use of clinical data. The study was approved by the Regional Committee for medical and health research ethics of Northern Norway (2018/1968/REK nord), and by the Data Protection Officer at UNN. The Norwegian version of SF-36 v. 1 was freely distributed by Knowledge Centre for the Health Services, Norwegian Institute of Public Health [32]. Permission to use the INQoL [20], the PSQI [33], and the FSS [34], respectively, was obtained.

RESULTS

Participants

The response rates are shown in Fig. 1. The overall HRQoL response rate was 84/90 (93%). Of all HRQoL respondents, 59 (70%) participated all three times, 16 (19%) twice, and 9 (11%) participated only once. Baseline characteristics of the sample comprising 75 c.826 C > A homozygotes and nine c.826 C > A compound heterozygotes are provided in Table 1. The compound heterozygotes had comparable disease duration to homozygotes but were younger (median 31 vs 49 years, $p = 0.033$). The status regarding work, educational achievement, ADL, ambulation, PAP therapy, and PEG, was relatively poorer among compound heterozygotes than homozygotes, whereas the frequency of cardiac involvement was comparable and the consumption of pain medication

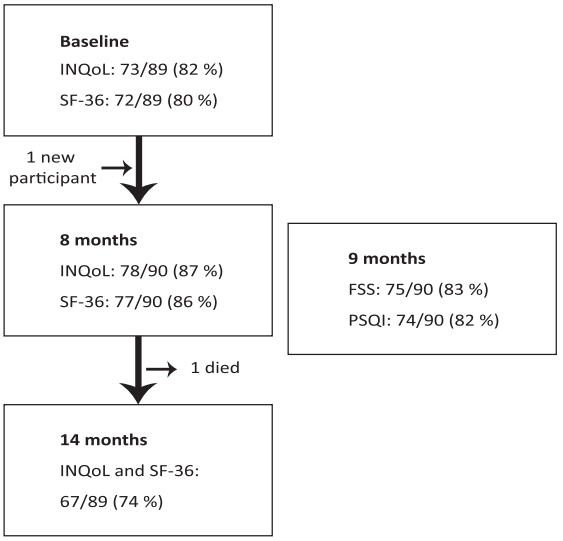

Fig. 1. Flow chart.

relatively higher among homozygotes. The subgroup of homozygotes was gender balanced, and males and females had comparable age and disease duration. Among homozygotes, females showed relatively poorer ADL and ambulation, a higher rate of PAP therapy, and a greater consumption of pain medication compared to males, whereas a higher proportion of males reported cardiac involvement. Comparison of compound heterozygous males and females was not applicable due to low sample size, heterogeneous genotypes, and unequal age distributions (Table 1 and 2).

Internal validity

Global scores for the PSQI, the FSS, and the HRQoL could be calculated for 89%, 97%, and 96–100% of the responses, respectively. SF-36 showed considerable floor effects on physical functioning, ceiling effects on social relationships, and both floor- and ceiling effects on role physical and role emotions (Table 3). The INQoL scale was reverse scored and had negligible ceiling effects but considerable floor effects on myalgia, fatigue, and dysphagia (Table 4). ICCs were moderate to high (0.62–0.85) on all SF-36 subscales except role emotions and change (Table 3). INQoL showed overall relatively higher ICCs (0.73–0.91) (Table 4) indicating higher rank-order stability across time. There were moderate to strong correlations between SF-36 and INQoL subscales sharing conceptual similarity (Table 5).

HRQoL over time

The measures of HRQoL at the three time points showed comparable mean scores (Table 3 and 4). The greatest difference between the highest and the lowest mean score was found on the subscales of bodily pain (4.9 points) and vitality (6.5 points) of the SF-36 (Table 3) and, correspondingly, myalgia (5.9 points) and fatigue (6.8 points) of the INQoL (Table 4). The change in the INQoL subscale scores from baseline to 14 months showed, in homozygotes, a worsening in muscle weakness and the INQoL index and a borderline significant worsening in activities (Table 4). Correspondingly, GLMM regression, including all three measurements of the homozygotes, showed an increase in muscle weakness (Table 6A), and an incremental tendency in the INQoL index ($p = 0.08$) (Table 6B). Five compound heterozygotes, all wheelchair dependent, completed the INQoL at baseline and 14 months. They showed a tendency towards worsening in muscle weakness but, more strongly, an improvement of the INQoL index (Table 4). On the particular SF-36 item concerning change in general health over the past year, worsening was reported by 42–57% of the homozygotes at the three respective measurements, compared to 17–29% of the compound heterozygotes. Among the homozygotes, 2–9% reported severe worsening, and 4–12% reported improvement. None of the compound heterozygotes reported severe worsening or any improvement.

HRQoL compared to normative data, and gender differences

Compared to the SF-36 normative data, the present study population showed significant impairments on all subscales except bodily pain, and the impairment of mental health in males was borderline significant (Table 3). Females showed relatively poorer scores both in the reference- and the present study population (not shown), but the female impairments were more pronounced among our participants compared to reference population (Table 3). Overall, homozygous females reported lower levels of HRQoL than homozygous males (Fig. 2A) and significantly on bodily pain, vitality, and social relationships (Table 3). Moreover, they showed a higher burden on several aspects of INQoL (Fig. 2B) and significantly on activities and independence (Table 4). GLMM analyses, including all three measurements of the homozygotes, showed that females tended

Table 1
Sociodemographic and clinical background data in all participants

	FKRP c.826 C > A homozygotes			*FKRP* c.826 C > A compound heterozygotes		
	All	Females	Males	All	Females	Males
	M (IQR); n/n (%)	M (IQR); n/n (%)	M (IQR); n/n (%)	M (IQR); n/n (%)	M (IQR); n/n (%)	M (IQR); n/n (%)
N	75/75 (100)	35/75 (47)	40/75 (53)	9/9 (100)	5/9 (56)	4/9 (44)
Age (years)	49 (33–58)	47 (33–57)	50 (34–61)	31 (28–36)	31 (28–35)	33 (25–56)
Range	16–75	16–71	16–75	25–63	25–35	25–63
Disease duration (years)	31 (18–47)	30 (19–45)	32 (18–49)	27 (16–33)	27 (16–30.5)	29 (15–55)
Spouse/ cohabitant *(Age ≥ 25 years)*	43/67 (64)	20/31 (64)	23/36 (64)	4/9 (44)	2/5 (40)	2/4 (50)
Living alone	23/72 (32)	10/34 (29)	13/38 (34)	2/9 (22)	1/5 (20)	1/4 (25)
ADL						
Independent	31/75 (41)	11/35 (31)	20/40 (50)	2/9 (22)	1/5 (20)	1/4 (25)
P.A.	15/75 (20)	11/35 (31)	4/40 (10)	6/9 (67)	4/5 (80)	2/4 (50)
Home care	3/75 (4)	3/35 (9)	0/40 (0)	0/9 (0)	0/5 (0)	0/4 (0)
Institution	2/75 (3)	2/35 (6)	0/40 (0)	0/9 (0)	0/5 (0)	0/4 (0)
Work/studies						
Yes, 100%	26/61 (43)	9/30 (30)	17/31 (55)	1/8 (13)	1/5 (20)	0/3 (0)
Partial incapacity	13/61 (21)	8/30 (27)	5/31 (16)	0/8 (0)	0/5 (0)	0/3 (0)
100% incapacity	19/61 (31)	11/30 (37)	8/31 (26)	6/8 (75)	3/5 (60)	3/3 (100)
Incapacity, other cause *(Age 18-67 years)*	2/61 (3)	1/30 (3)	1/31 (3)	1/8 (13)	1/5 (20)	0/3 (0)
Higher education[a] *(Age ≥ 25 years)*	37/68 (54)	18/32 (56)	19/36 (53)	3/9 (33)	3/5 (60)	0/4 (0)
Ambulation						
Independent	40/75 (53)	13/35 (37)	27/40 (68)	3/9 (33)	1/5 (20)	2/4 (50)
Walking aids	13/75 (17)	7/35 (20)	6/40 (15)	0/9 (0)	0/5 (0)	0/4 (0)
Wheelchair	22/75 (29)	15/35 (43)	7/40 (18)	6/9 (67)	4/5 (80)	2/4 (50)
PAP						
All	22/75 (29)	15/35 (43)	7/40 (18)	3/9 (33)	1/5 (20)	2/4 (50)
Day and night	2/75 (3)	2/35 (13)	0/40 (0)	3/9 (33)	1/5 (20)	2/4 (50)
CM	16/73 (22)	4/34 (12)	12/39 (31)	2/9 (22)	1/5 (20)	1/4 (25)
PEG	1/75 (1)	1/35 (3)	0/40 (0)	3/9 (33)	1/5 (20)	2/4 (50)
Analgesics (myalgia)						
Never	51/75 (68)	18/35 (51)	33/40 (83)	4/8 (50)	1/5 (20)	3/3 (100)
Daily/almost daily	4/75 (5)	4/35 (11)	0/40 (0)	0/8 (0)	0/5 (0)	0/3 (0)

(a) ≥ 1 year in college or university. M = median, IQR = inter-quartile range, ADL = activities of daily living, P.A. = user-controlled personal assistance, PAP = Positive Airway Pressure support, CM = cardiomyopathy (self-reported), PEG = Percutaneous Endoscopic Gastrostomy.

to have more severe muscle weakness (Table 6A) and a significantly worse INQoL index than males (Table 6B).

HRQoL and genotypes

SF-36 showed no significant differences in HRQoL between the two genotype groups but a tendency towards poorer physical functioning, yet better social and mental HRQoL among the compound heterozygotes (Table 3, Fig. 2C). On INQoL, the compound heterozygotes reported significantly higher burden on the domains of dysphagia and independence (Table 4, Fig. 2D). The prevalence of dysphagia was 6/9 (67%) in compound heterozygotes compared to 14/80 (18%) in homozygotes.

HRQoL and age/ disease duration

In homozygotes, both age and disease duration correlated positively with the following INQoL subscales: muscle weakness ($p = 0.017$ and

Table 2
Genotypes and natural history of *FKRP* c.826 C > A compound heterozygous participants

No.	Genotype *FKRP* c.826 C > A /	Sex	Age (years)	Age at onset (years)	Age at W/C (years)	Age at CM (years)	Age at PAP (years)	PEG
1. [a]	962 C > A, (Ala321Glu)	F	30	12	23	24	–	–
2. [a]		F	35	8	20	–	–	–
3. [a†]		M	36	2	7	18	23	+
4.		F	34	3	14	31	27	+
5.		M	63	1	12	–	31	+
6.	899T > C, (Val300Ala)	F	25	11	–	–	–	–
7.	1323T > G (Phe441Leu)	F	31	1	28	28	–	–
8.	328 C > T, (Arg110Trp)	M	30	7	–	–	–	–
9.	166T > A, (Phe56Ile)	M	25	13	–	20	–	–

(a) Siblings, (†) died during the study. W/C = wheelchair dependency, CM = cardiomyopathy (here: LGMDR9-related abnormalities on echocardiography according to medical records), PAP = Positive Airway Pressure support, PEG = Percutaneous Endoscopic Gastrostomy, F = female, M = male. Adapted table from a previous study [2] (Table A).

Table 3
SF-36 measurements

	Baseline, 8 and 14 months			Baseline		First responses
SF-36 subscales	Scores (n = 66–76) Mean $_{SD}$	ICC	Floor/Ceiling %	Below reference All F /M (n = 36/36) p	Homoz. F/M (n = 31/34) p	Homoz/Comp (n = 75/9) p[a]
Physical functioning	$37.9_{34.1}$ $33.7_{32.0}$ $35.8_{34.4}$	0.85	19–23/ 3–6	*/ *	0.076 (F worse)	0.37 (Comp worse)
Role physical	$46.8_{43.9}$ $44.1_{41.8}$ $47.0_{43.3}$	0.61	33–39/ 28–35	*/ *	0.75 (F worse)	0.51 (Homoz worse)
Bodily pain	$68.0_{25.5}$ $66.4_{25.8}$ $71.3_{22.5}$	0.75	0/ 18–20	0.36 / - (above reference; 0.85)	**0.040 F worse**	0.56 (Comp worse)
General health	$48.8_{24.7}$ $47.3_{25.9}$ $48.6_{22.3}$	0.80	1–4/ 0–1	*/ *	0.18 (F worse)	1.0
Vitality	$44.5_{23.3}$ $45.2_{24.2}$ $51.0_{23.6}$	0.83	3–5/ 0	***/ 0.018**	**0.008 F worse**	0.85 (Homoz worse)
Social functioning	$67.0_{27.8}$ $66.9_{27.2}$ $66.6_{26.9}$	0.66	0–1/ 18–28	***/ 0.0005**	**0.025 F worse**	0.39 (Homoz worse)
Role emotional	$65.7_{40.6}$ $65.3_{40.7}$ $62.2_{43.8}$	0.48	19–27/ 52–57	*** / 0.0073**	0.11 (F worse)	0.55 (Homoz worse)
Mental health	$73.3_{16.7}$ $74.2_{18.3}$ $75.9_{16.4}$	0.79	0/ 0–1	**0.006**/ 0.050	0.18 (F worse)	0.24 (Homoz worse)
Change	$38.5_{18.3}$ $37.7_{20.1}$ $43.2_{18.9}$	0.45	2–8/ 1–4	N.D.	0.46 (F worse)	0.65 (Homoz worse)

(a) Age- and sex adjusted p-value. (*) $p < 0.0001$. P-values < 0.05 are highlighted. SD = standard deviation, ICC = intraclass correlation coefficient, homoz = *FKRP* c.826 C > A homozygotes, compound = *FKRP* c.826 C > A compound heterozygotes, F = females, M = males, N.D. = no data. The SF-36 subscales range 0–100 (lowest to highest HRQoL). Since only 7/9 compound heterozygotes participated at baseline, the first responses, rather than only the baseline responses, were included for comparison with homozygotes.

Table 4
INQoL measurements

INQoL domains	Baseline, 8 and 14 months			Baseline and 14 months		Baseline	First responses
	Scores (n = 67–73) Mean $_{SD}$	ICC	Floor/ Ceiling %	Increase Mean $_{SD}$ (median)		Homoz F/M (n = 31/35) p	Homoz/ Comp (n = 75/9) p[b]
				Homoz (n = 55)	Comp[a] (n = 5)		
Weakness	$62.1_{24.5}$ $63.0_{23.4}$ $64.5_{23.9}$	0.91	5–7/ 1–5	$4.7_{9.8}$ (5.3) **(p = 0.001)**	$2.1_{13.7}$ (5.3) (p = 0.49)	0.39 (F worse)	0.11 (Comp worse)
Myalgia	$29.8_{26.2}$ $31.0_{27.3}$ $25.1_{26.0}$	0.81	30–36/ 0	$-2.0_{17.9}$ (0) (p = 0.41)	$-3.0_{4.7}$ (0) (p = 0.18)	0.38 (F worse)	0.89 (Comp worse)
Fatigue	$39.9_{28.0}$ $43.2_{28.3}$ $36.4_{31.5}$	0.81	19–33/ 1–3	$-1.0_{18.2}$ (0) (p = 0.68)	$6.3_{23.1}$ (0) (p = 0.59)	0.71 (F worse)	0.62 (Comp worse)
Dysphagia	$8.2_{16.7}$ $7.8_{15.0}$ $4.8_{12.0}$	0.81	11–82/ 0	$-0.2_{8.7}$ (0) (p = 0.87)	$-11.6_{14.1}$ (–5.3) (p = 0.11)	0.51 (F worse)	**0.004 Comp worse**
Activities	$55.8_{23.4}$ $59.9_{23.8}$ $57.0_{24.7}$	0.91	0–1/ 0–3	$3.3_{11.7}$ (1.9) (p = 0.051)	$0.9_{13.7}$ (–0.9) (p = 0.89)	**0.044 F worse**	0.14 (Comp worse)
Indepen-dence	$40.9_{29.7}$ $43.6_{30.5}$ $41.2_{29.5}$	0.91	9–10/ 1–3	$1.3_{14.9}$ (0) (p = 0.52)	$-1.1_{1.5}$ (0) (p = 0.16)	**0.004 F worse**	**0.0002 Comp worse**
Relationships	$34.2_{22.1}$ $35.0_{21.0}$ $34.0_{21.4}$	0.73	7–8/ 0	$1.4_{18.4}$ (0) P = 0.57)	$5.6_{20.4}$ (–2.3) (p = 1.0)	0.73 (F worse)	0.64 (Homoz worse)
Emotions	$38.1_{21.5}$ $39.4_{23.5}$ $34.5_{20.9}$	0.81	3–8/ 0	$0.3_{14.6}$ (0) (p = 0.88)	$-6.1_{14.6}$ (–5.6) (p = 0.36)	0.064 (F worse)	0.30 (Homoz worse)
Body image	$43.0_{26.0}$ $44.9_{25.9}$ $44.2_{26.0}$	0.74	3–9/ 0–3	$2.9_{20.2}$ (0) (p = 0.29)	$-6.1_{23.7}$ (0) (p = 1.0)	0.40 (F worse)	0.15 (Comp worse)
INQoL index	$47.3_{18.0}$ $48.3_{18.0}$ $48.2_{19.6}$	0.81	0–2/ 0	$4.8_{12.3}$ (3.9) **(p = 0.010)**	$-4.4_{9.1}$ (–6.1) (p = 0.34)	0.15 (F worse)	1.0

(a) Patient No. 1, 2, and 5–7, Table 2, (b) Age- and sex-adjusted p-value. P-values < 0.05 are highlighted. SD = standard deviation, ICC = intraclass correlation coefficient of repeated measurements homoz = *FKRP* c.826 C > A homozygotes, compound = *FKRP* c.826 C > A compound heterozygotes, F = females, M = males. The INQoL domain scores range 0–100 (no burden to maximal burden). Since only 7/9 compound heterozygotes participated at baseline, first response of each individual was included for comparison with homozygotes. Four zero-scores of muscle weakness out of 218 registrations were not included due to clear inconsistency from the other registrations of the respective respondent.

Table 5
Bivariate correlations between selected subscales from INQoL and SF-36

INQoL	SF-36	r_s (n = 69–72)	95% CI
Muscle weakness	Physical functioning	–0.56 *	(–0.71, –0.37)
Fatigue	Vitality	–0.70 *	(–0.81, –0.55)
Myalgia	Bodily pain	–0.77 *	(–0.86, –0.66)
Activity	Physical functioning	–0.64 *	(–0.76, –0.46)
	Role physical	–0.44 *	(–0.62, –0.23)
Independence	Physical functioning	–0.69 *	(–0.80, –0.54)
Relationships	Social functioning	–0.73 *	(–0.83, –0.60)
Emotions	Mental health	–0.67 *	(–0.79, –0.51)
	Role emotional	–0.49 *	(–0.65, –0.29)

(*) p < 0.001, r_s = Spearman´s rho. Note: INQoL and SF-36 have inverse directions on the scales, hence negative correlation coefficients.

Table 6
Multivariate regression models (generalized linear mixed models) of INQoL scores

Independent variables	p[a]	Beta	*p*	95% CI
A) Muscle weakness (c.826 C > A homozygotes)				
Age (years)	0.01	0.44	0.008	(0.1, 0.8)
Female (vs male)	0.21	7.19	0.14	(–2.3, 16.6)
Time (months)	0.002	–	0.003	–
0		–4.22	0.001	(–6.8, –1.6)
8		–3.50	0.003	(–5.8, –1.2)
14		–	–	–
B) INQoL index (c.826 C > A homozygotes)				
Age (years)	0.04	0.26	0.03	(–0.03, 0.50)
Female (vs male)	0.03	9.18	0.02	(1.6, 16.8)
Time (months)	0.07	–	0.08	–
0		–3.57	0.03	(–6.73, –0.41)
8		–1.46	0.29	(–4.15, 1.24)
14		–	–	–
C) INQoL index (All participants)				
Age (years)	0.02	0.001	0.99	(–0.20, 0.21)
Female (vs male)	0.06	5.25	0.06	(–0.23, 10.74)
Higher education (vs non)	0.14	4.08	0.14	(–1.28, 9.44)
Weakness (0–100)	<0.001	0.37	<0.001	(0.25, 0.49)
Fatigue (0–100)	<0.001	0.10	0.006	(0.03, 0.18)
Myalgia (0–100)	<0.001	0.04	0.47	(–0.06, 0.13)
Dysphagia (0–100)	0.17	–0.03	0.75	(–0.22, 0.16)
PAP (vs non)	0.045	1.15	0.70	(–4.80, 7.09)
Time	0.13	–	0.84	–

14 months = reference category for the variable Time. p^a = *p*-value before mutual adjustment for the other independent variables. CI = confidence interval, PAP = Positive Airway Pressure support. Dependent variable: (A) Perceived muscle weakness from *FKRP* c.826 C > A homozygotes. (B) The INQoL index from *FKRP* c.826 C > A homozygotes. (C) The INQoL index from all participants. Variables not included in Table 6C due to a $p^a \geq 0.20$ were genotype ($p^a = 0.30$), cardiomyopathy ($p^a = 0.60$), and PSQI ($p^a = 0.33$). Level of ambulation was significant ($p^a < 0.001$) but not included due to substantial similarity with muscle weakness.

$p = 0.004$), fatigue ($p = 0.038$ and $p = 0.004$), dysphagia ($p = 0.030$ and $p < 0.001$), activities ($p = 0.036$ and $p = 0.011$), independence ($p = 0.002$ and $p < 0.001$), and the INQoL index ($p = 0.039$ and $p = 0.039$) ($r_s = 0.24$–0.44). Duration was also positively correlated with the subscale of relationships ($r_s = 0.31$, $p = 0.008$). Myalgia, emotions, and body image did not correlate with age or disease duration in homozygotes. In compound heterozygotes, age and disease duration did not correlate with any of the INQoL subscales.

HRQoL and LGMDR9-related symptoms/ involvements

The frequency of any level of muscle weakness was 93–97%, fatigue 67–82%, myalgia 63–68%, and dysphagia 17–28% at the three respective measurements. Multivariate regression showed that muscle weakness and fatigue were independent predictors of the INQoL index (Table 6C). Comparing of the INQoL index of the independent walkers and the wheelchair and walking aid subgroups showed that the INQoL index was associated with wheelchair dependency ($p = 0.002$) and more strongly with walking aids ($p < 0.0001$) but did not differ significantly between the wheelchair and walking aid subgroups ($p = 0.10$).

Fatigue and subjective sleep quality

The FSS cut-off score gave a fatigue prevalence of 41% (14/34) in females and 38% (15/39) in males. FSS correlated positively with age, PSQI, and the INQoL domains of emotions, myalgia, and muscle weakness but was unrelated to sex. Only emotions ($p = 0.007$) and myalgia ($p = 0.009$) were identified as independent correlates (Supplementary Table 1). Median FSS was relatively higher in those using walking aids (5.3) compared to wheelchair (4.2) and independent walkers (4.3), but the subgroup differences were not significant. FSS

correlated strongly with the fatigue scale of INQoL ($r_s = 0.76$, $p < 0.0001$), moderately with the INQoL index ($r = 0.46$, $p < 0.0001$) and, as expected, negatively with the vitality scale of SF-36 ($r_s = -0.68$, $p < 0.0001$).

Applying the PSQI cut-off score, 19/34 (56 %) of females and 21/37 (43 %) males were classified as poor sleepers. PSQI was related to myalgia ($p = 0.003$) but not age, sex, PAP therapy, wheelchair dependency, or the INQoL domains of muscle weakness or emotions (Supplementary Table 2).

HRQoL and socideomographic variables

The proportion of participants 30–69 years old living alone was 18/62 (29%), which is comparable to corresponding age groups in the general population (21–26%) [30]. Among participants age 25–39 years,

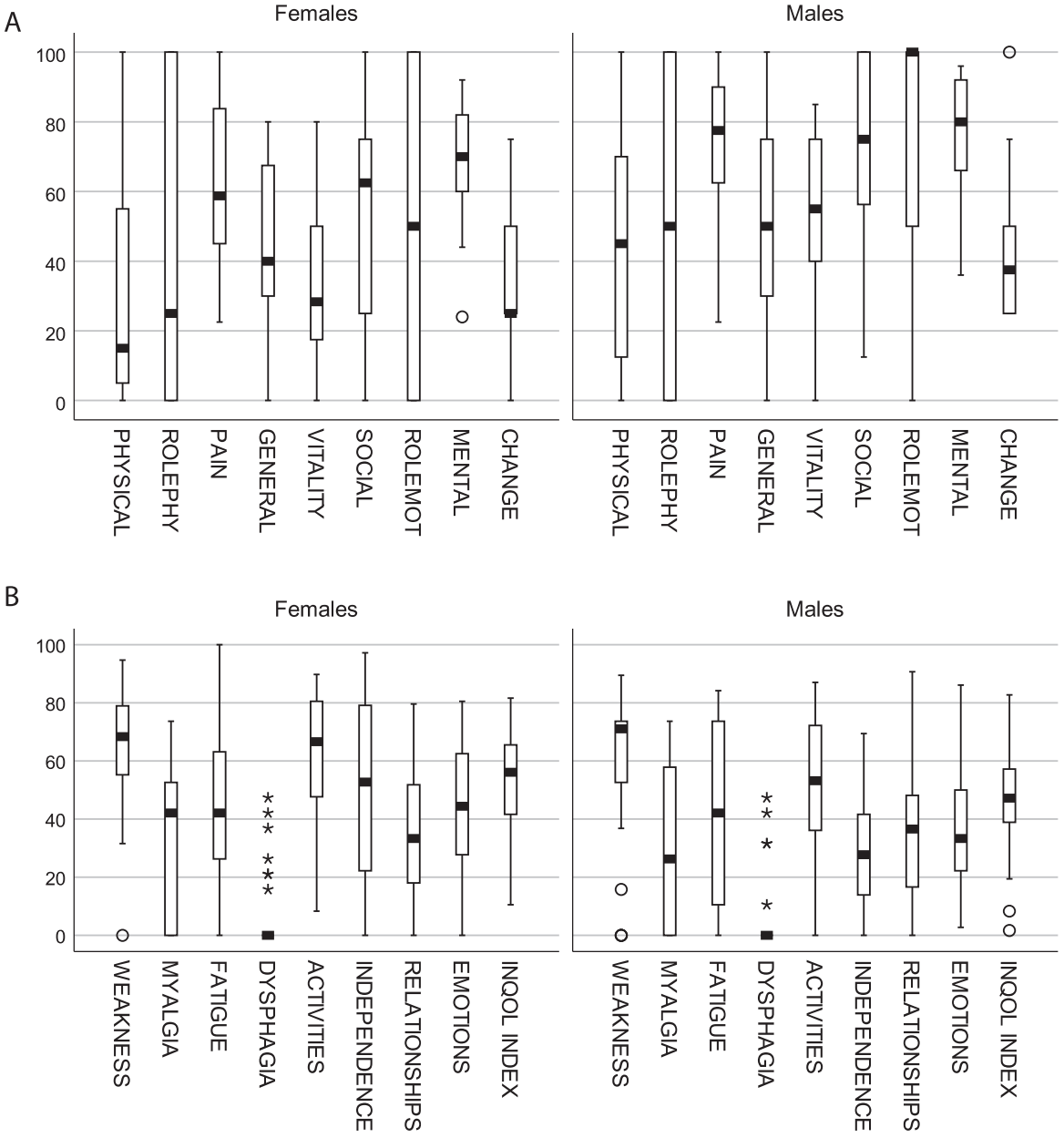

Fig. 2. (*Continued*)

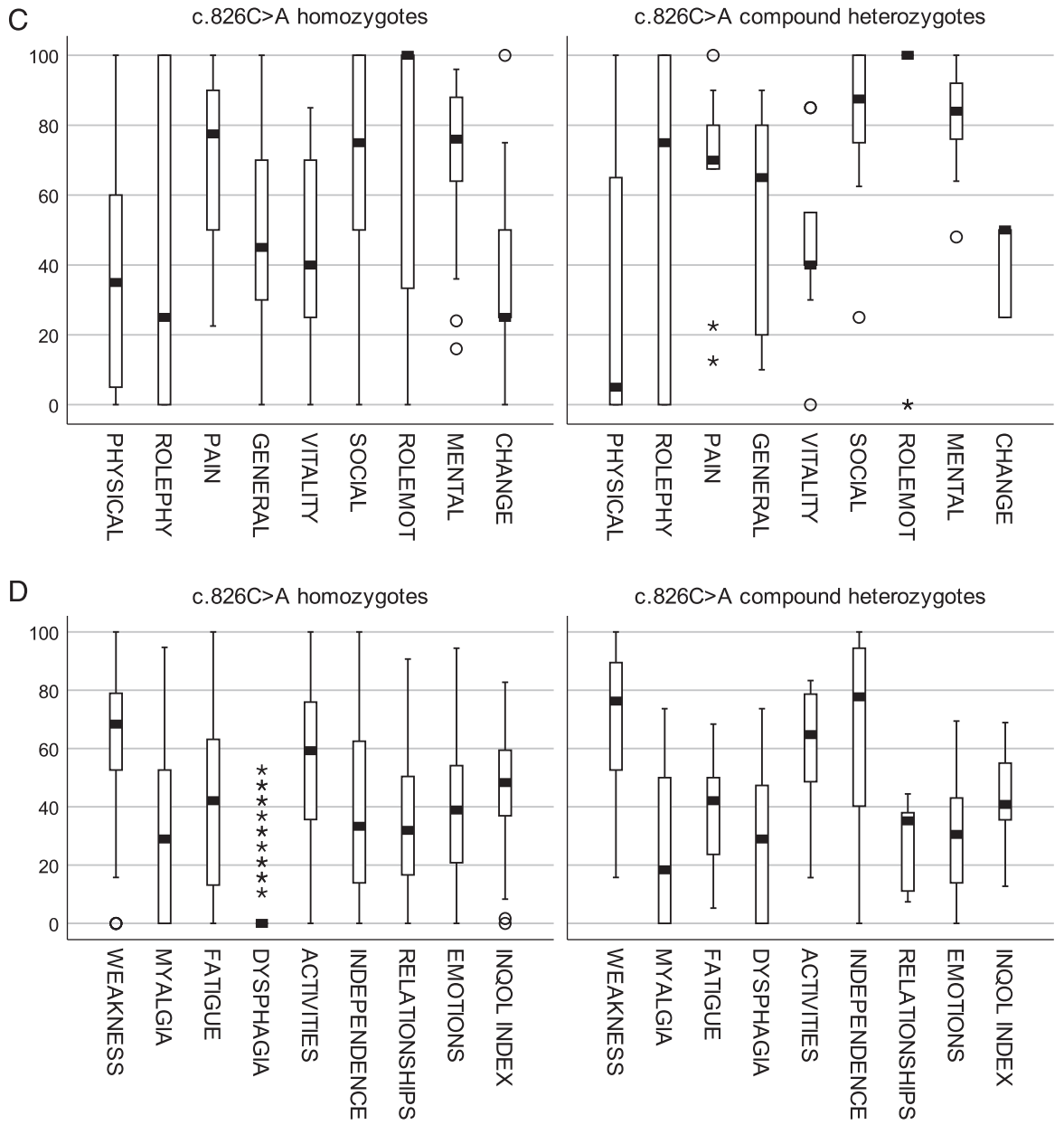

Fig. 2. Box plot showing (A) SF-36 scores in *FKRP* c.826 C > A homozygous females vs males. (B) INQoL scores in *FKRP* c.826 C > A homozygous females vs males. (C) SF-36 scores in *FKRP* c.826 C > A homozygotes vs c.826 C > A compound heterozygotes. (D) INQoL scores in *FKRP* c.826 C > A homozygotes vs c.826 C > A compound heterozygotes. Notes: SF-36 scales range from lowest (0) to highest (100) HRQoL. The INQoL subscales range from no (0) to maximal (100) burden. *FKRP* c.826 C > A compound heterozygotes are significantly younger than c.826 C > A homozygotes (Table 1).

9/14 (64%) females and 5/14 (36%) males had higher education, which are relatively similar to the 60% of females and 40% of males reported in the general population [30].

The participants living alone did not differ on any aspect of HRQoL from those living with a partner or family. Nevertheless, there was a negative trend between living alone and most aspects of HRQoL except independence. In participants 25+ years, those with higher education showed poorer HRQoL on mental health ($p = 0.047$) and marginally for social function ($p = 0.068$) as well. The INQoL index was unrelated to educational achievement (Table 6C). Median age of participants younger than 67 years

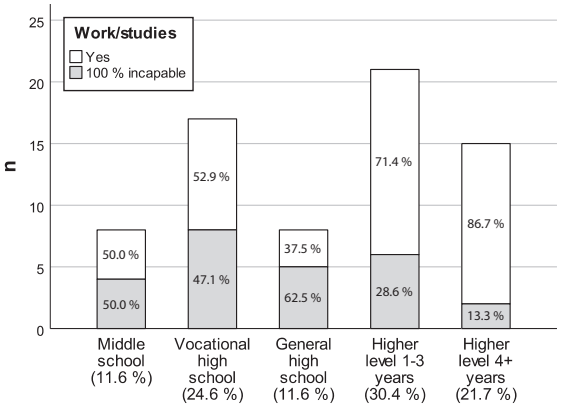

Fig. 3. Bar chart showing the distribution of participants 25–67 years old with 100% long-term sickness absence or incapacity for work or studies, according to the highest educational level achieved ($n = 69$).

with long-term or permanent 100% incapacity for work or studies was 54.5 years (range 24–66). In the age group 25–67 years, 100% study/work incapacity was associated with lower level of education (OR 3.72, 95% CI: 1.31–10.53, $p = 0.011$) (Fig. 3), more severe bodily pain ($p = 0.021$) and myalgia ($p = 0.007$), and poorer general health ($p = 0.035$), role emotions ($p = 0.018$), activities ($p = 0.006$), and independence ($p < 0.001$). However, the INQoL index was unrelated to study/work incapacity.

Supportive care

In total, 17/40 (43%) females and 21/44 (45%) males received physiotherapy treatment regularly. Reasons provided for not having physiotherapy included absent treatment effect (15/46), absent needs (11/46), costs (8/46), school/work (6/46), lack of available services (4/46), transportation/ travel difficulties (3/46), and 13/46 reported other various factors. Mental health treatment was received by 3/40 females and 3/43 males while 9/40 females and 4/43 males did not receive although they reported needs for mental health treatment.

DISCUSSION

This was an exploratory study of HRQoL in a Norwegian sample with LGMDR9 comprising 75 *FKRP* c.826 C > A homozygotes and nine *FKRP* c.826 C > A compound heterozygotes. We found that both physical, social, and emotional aspects of HRQoL are compromised. Over 14 months, the homozygotes showed worsening in perceived weakness and disease burden and tentatively in activities, whereas compound heterozygotes had a tendency towards worsening in weakness but surprisingly some relief in their disease burden. Compound heterozygotes reported a higher burden on dysphagia and physical aspects of INQoL compared to homozygotes. Among homozygotes, females reported poorer HRQoL on aspects of bodily pain, social relationships, and emotions and a higher physical burden and overall burden compared to males. Disease burden was predicted by perceived muscle weakness and fatigue. Fatigue was prevalent and related to myalgia and emotions. Poor sleep was also frequent and related to myalgia.

Despite only a 14-month follow-up period, we observed a subjective worsening in muscle weakness and disease burden among c.826 C > A homozygotes. This was unexpected considering the slowly progressive nature of LGMDR9 in general and for this genotype in particular [5]. The paradoxical tendency towards a relief in burden of disease among the c.826 C > A compound heterozygotes, who generally have a more progressive phenotype, is likely related to the late disease stage of the participants. All compound heterozygous participants in the prospective study had entered a wheelchair-dependent stage, where progression may not be that easily perceived or stressful as before transition to wheelchair. This corresponds with the findings of a relatively higher disease burden and levels of fatigue in subjects with walking aids compared to wheelchair-dependent subjects. Furthermore, compared to homozygotes, compound heterozygotes also showed tendencies towards better scores on mental and social aspects despite significantly worse physical scores. These observations align with the "disability paradox", which is the phenomenon where the perceived burden is disproportionate to the level of disability because of mental and environmental adaptations [35].

The relatively poorer HRQoL observed in females corresponds with previous studies on muscle diseases [11] but also with overall tendencies observed in normative data [28, 36]. Background clinical characteristics suggest that sex differences in disease involvement may be a contributing factor. Similar sex differences were also evident and discussed in our natural history study [2] but need further validation. Nevertheless, regardless of the causality, the current findings indicate more unmet care needs among females.

Most domains of INQoL worsened by age and disease duration. This highlights the importance

of preventive and promotional care. Myalgia, body image, and emotions were unrelated to age and duration, suggesting that the potential burden of these aspects should be addressed at an early stage in the treatment.

Fatigue was predictive of disease burden, which concurs with published findings in other muscle diseases [11, 13, 37]. Applying the FSS cut-off value of ≥ 5, 40% of the patients had clinical levels of fatigue, which is relatively high compared to 23.1% reported in a previous Norwegian general population study [27]. Fatigue is a heterogeneous and multifactorial phenomenon [38]. In our sample, fatigue severity was related to myalgia and negative emotions. In the published literature, there are strong evidence for an interrelationship between fatigue, musculoskeletal pain, and psychological distress and explanatory theories include neurotransmitter imbalances, hypothalamic-pituitary-adrenal axis dysregulation, inflammation, central sensitization, and genetic factors [39]. A previous prospective observational study on ambulatory patients with NMD suggested that fatigue also relates to the decline in physical activity that follows the progressive weakness [40]. Currently, there are accumulating evidence that moderate-intensity aerobic exercise in NMD may alleviate fatigue, and even some evidence of disease-modifying effects [41, 42], and anti-inflammatory and epigenetic mechanisms have been suggested [43]. Our findings suggest that myalgia and mental distress should be addressed in fatigued patients and both cognitive behavioral therapy and exercise therapy could be relevant approaches. However, NMD seem to respond differently to exercise and long-term data are limited [43, 44]. In this regard, both studies on short- and long-term effects of exercise therapy and comparative trials on fatigue management in LGMDR9 are required to provide evidence-based recommendations.

Myalgia was frequently reported. Nevertheless, bodily pain was comparable to the reference population, the reported consumption of pain medication for myalgia was low, and the INQoL index was unrelated to myalgia. Comparatively, in a study of Facioscapulohumeral muscular dystrophy (FSHD), a related condition well known to be associated with pain, the burden of myalgia was relatively higher (median 44.8 vs mean 25.1–31.0 of 100) and affected the INQoL index [45]. Myalgia did thus not emerge as a major issue in our LGMDR9 cohort, which is contrary to a previous study [12]. However, myalgia was associated with fatigue and poor sleep, in which myalgia may play a perpetuating role.

Subjective sleep quality did not impact disease burden. Nevertheless, the high frequency of poor-quality sleep suggests that sleep may be an overlooked aspect of care. The frequency in females (56%) and males (43%), respectively, was relatively high compared to general population studies in Germany (42.5% of females and 28.7% of males) [46] or Austria (36.5 % of females and 26.6 % of males) [47]. The overall frequency (49%) was comparable to findings in FSHD (50%) [24] and adult patients with Duchenne muscular dystrophy (55%) [25]. Although we identified a relationship with myalgia, poor sleep is unspecific and may have various underlying issues and sleep disorders that should be addressed.

The additional finding of relatively lower levels of long-term sickness absence or incapacity among participants with higher education aligns with the general pattern [48, 49]. Lastly, there were some limitations in the access to qualified physiotherapy and mental health care. These are also potential areas for improvement.

Strengths and limitations

We believe the findings are representative of the Norwegian LGMDR9 population 16+ years given the nation-wide recruitment, high response rates, and gender balance. Measurement at three time points provided insight into the longitudinal stability and change at cohort and individual level. INQoL provided information about expression of disease-related symptoms, burden, and relationships with burden. Our analyses indicated good internal validity. The use of SF-36 enabled comparison with a reference population, which was useful in the interpretations of the INQoL with regard to impaired areas of HRQoL.

This study also has some important limitations. Due to the exploratory design we accepted a higher risk of conducting type I error (false positive) and urge for caution in interpreting *p*-values particularly in the zone of 0.01 to 0.05 as significant. Findings that are flagged as significant should be replicated in future studies. The sample size of c.826 C > A compound heterozygotes was low, which clearly limits the statistical power. The study was conducted during the COVID-19 pandemic, which may have introduced period-specific results generalizing less well to post-COVID life. The increase in perceived muscle weakness observed in homozygotes could potentially result from inactivity due to restrictions [50]. Nevertheless, since all data collections were per-

formed in comparable and relatively COVID-19 free periods, the cross-sectional measurements were comparable, and no longitudinal change was detected in the aspects of relationships, myalgia, fatigue, or emotions at the individual level, we believe the pandemic had minor effects on the results. Lastly, interpretations of the prospective study were also hampered by the fact that INQoL has not been validated on sensitivity in LGMD. Neither a 6-year follow-up study of patients with Dystrophia myotonica type 1 [51] nor a 20-month follow-up study of patients with Oculopharyngeal muscular dystrophy [52] detected deterioration in the INQoL measurements despite objective progression of muscle weakness.

Conclusions

This study provided insight into the impacts of LGMDR9 on HRQoL, subjective changes over a 14-months period, and relationships with disease burden. Both physical, emotional, and social aspects of HRQoL were impaired, which advocates for a multi-disciplinary care. In c.826 C > A homozygotes, perceived muscle weakness and disease burden deteriorated during the study, which is relevant information for clinical trials. However, validation of sensitivity of the INQoL measure in LGMD remains and potential COVID-19 effects should be considered. Among homozygotes, females reported a higher burden, which indicates the need for a gender-specific perspective in treatment and care. Muscle weakness and fatigue proved particularly important where fatigue is amenable to treatment. Associations suggest that myalgia and mental distress are potential targets for the treatment of fatigue, but further studies are needed to establish evidence for effective interventions. Poor sleep was frequent and will be investigated further. The combined use of a generic and a disease-specific HRQoL is particularly useful for research purposes. However, a generic HRQoL that is brief to minimize patient burden and optimize response rate, constructed for utility analyses, and with accessible norms may be preferable. Adding an item of overall QoL or happiness would have provided insight into the significance of the disease in the patient´ lives as a whole and can be considered in future research.

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CONFLICT OF INTEREST

The authors have no conflict of interest to report.

SUPPLEMENTARY MATERIAL

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Paper III

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ORIGINAL COMMUNICATION

Insomnia and sleep-disordered breathing in FKRP-related limb-girdle muscular dystrophy R9. The Norwegian LGMDR9 cohort study (2020)

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Abstract

Limb-girdle muscular dystrophy R9 (LGMDR9) is a progressive and disabling genetic muscle disease. Sleep is relevant in the patient care as it impacts on health, functioning, and well-being. LGMDR9 may potentially affect sleep by physical or emotional symptoms, myalgia, or sleep-disordered breathing (SDB) through cardiorespiratory involvement. The objective was to investigate the occurrence of insomnia and unrecognized or untreated SDB in LGMDR9, associated factors, and relationships with fatigue and health-related quality of life (HRQoL). All 90 adults in a Norwegian LGMDR9 cohort received questionnaires on sleep, fatigue, and HRQoL. Forty-nine of them underwent clinical assessments and 26 without mask-based therapy for respiration disorders additionally underwent polysomnography (PSG) and capnometry. Among 77 questionnaire respondents, 31% received mask-based therapy. The prevalence of insomnia was 32% of both those with and without such therapy but was significantly increased in fatigued respondents (54% vs 21%). Insomnia levels correlated inversely with mental HRQoL. Among 26 PSG candidates, an apnea–hypopnea index (AHI) ≥ 5/h was observed in 16/26 subjects (≥ 15/h in 8/26) with median 6.8 obstructive apneas and 0.2 central apneas per hour of sleep. The AHI was related to advancing age and an ejection fraction < 50%. Sleep-related hypoventilation was detected in one subject. Fatigue severity did not correlate with motor function or nocturnal metrics of respiration or sleep but with Maximal Inspiratory Pressure (r = − 0.46). The results indicate that insomnia and SDB are underrecognized comorbidities in LGMDR9 and associated with HRQoL impairment and heart failure, respectively. We propose an increased attention to insomnia and SDB in the interdisciplinary care of LGMDR9. Insomnia and pulmonary function should be examined in fatigued patients.

Keywords Muscular dystrophies, limb-girdle · Sleep initiation and maintenance disorders · Respiration disorders, sleep apnea syndromes · Sleep · Fatigue

Introduction

Limb-girdle muscular dystrophy type R9 (LGMDR9) is a rare autosomal recessive muscle disease caused by pathogenic variants in the fukutin-related protein (FKRP) gene. LGMDR9 is characterized by slowly progressive proximal weakness and may be accompanied by cardiomyopathy and/or ventilatory failure [1–3]. Currently, there is no causal treatment available. Clinical management is interdisciplinary, focusing on supporting cardiorespiratory function,

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preventing complications, and optimizing daily functioning and health-related quality of life (HRQoL). Sleep affects overall health and HRQoL [4]. A previous study on the Norwegian LGMDR9 cohort (Jensen SM et al., submitted paper) showed that HRQoL was impaired and subjective sleep disturbance more frequent compared to studies on general populations. Additionally, fatigue was prevalent and closely related to disease burden. According to an explanatory model in neuromuscular disorders (NMD), sleep disturbance, together with physical inactivity and pain, may act as a perpetuating factor of fatigue [5]. Sleep may thus be an area that needs increased clinical attention in LGMDR9. Sleep disturbance is unspecific but may represent sleep disorders with specific treatment options such as insomnia and sleep-related breathing disorders (sleep-disordered breathing, SDB), which both are relevant in NMD.

Insomnia disorders involve nighttime problems of initiation or maintenance of sleep or early-morning awakening that causes daytime impairment or dissatisfaction with sleep [6]. Insomnia relates to various somatic and mental health conditions [7]. First-line treatment is Cognitive Behavioral Therapy for Insomnia (CBT-I) and both physical and digital CBT-I have been found effective [7]. Studies on patients with comorbid insomnia and sleep apnea (COMISA) have shown that CBT-I with an adapted and interdisciplinary approach may also improve tolerance and adherence to mask-based therapy [8]. In Duchenne muscular dystrophy, the increased risk of insomnia and its potentially negative influence on essential non-invasive ventilation (NIV) and HRQoL have been recognized and CBT-I or sleep hygiene advices are recommended interventions [9]. A recent study on milder muscular dystrophies including a subgroup with LGMD reported that insomnia and fatigue are related [10]. We are not aware of any other studies of insomnia in patients with LGMD. The impact of insomnia in NMD in general seems to be understudied.

SDB includes sleep-related hypoventilation and hypoxemia, obstructive sleep apnea (OSA), and central sleep apnea (CSA) [6]. NMD may cause hypoventilation by respiratory muscle weakness, scoliosis, stiffening of the chest wall, and subsequent intrapulmonary changes [11]. Furthermore, NMD may promote OSA by pharyngeal hypotonia, macroglossia, or possibly by collapsibility of the upper airways due to low lung volumes and may promote CSA by cardiomyopathy or hypoventilation [12]. Hypoventilation during rapid eye movement (REM) sleep is recognized as the earliest sign of respiratory failure [12], which may be related to sleeping position, impairment of chemosensitivity during sleep, and physiological REM sleep atonia [13, 14]. Mask-based therapies may provide efficient support for SDB, whereas inappropriate treatment can have aggravating effects [14]. In Duchenne muscular dystrophy and amyotrophic lateral sclerosis, which are more rapidly progressive diseases, non-invasive ventilation (NIV) has also been shown to prolong survival and improve HRQoL [14, 15]. In LGMD, respiratory involvement is rather unexplored and no disease-specific respiratory guidelines exist.

Our previous study of natural history in the Norwegian LGMDR9 cohort [3] showed that indication for mask-based therapy was restrictive pulmonary function alone in 47%, OSA alone in 22%, and both combined in 31%. Initiation of mask-based therapy was usually preceded by wheelchair dependency except in those who only had OSA. Females were more prone to become wheelchair dependent and require mask-based therapy, whereas males were more predisposed to cardiomyopathy. The level of respiratory follow-up was variable, which means that SDB may be underrecognized.

In the present study, we assessed the prevalence and levels of insomnia and unrecognized or untreated SDB in the Norwegian LGMDR9 cohort and examined relationships with demographic and clinical variables and indicators of HRQoL, particularly fatigue. We also examined whether fatigue in people with LGMDR9 relates to pulmonary function since respiratory muscle weakness and chest wall changes tend to increase the work of breathing. More knowledge about these issues may optimize the clinical management of this patient group.

Methods

Participants

Previously, we identified 153 individuals (135 adults, ≥ 16 years) with a genetically confirmed LGMDR9 in Norway [3]. They were all invited to participation in «The Norwegian LGMDR9 cohort study» at the National Neuromuscular Centre, Norway (NMK), University Hospital of North Norway (UNN). Participants provided clinical information by completing a project-specific questionnaire and by consenting to retrieve patient notes from the specialist centers. All adult participants in the cohort study were invited to respectively a sleep survey, a HRQoL survey, and a clinical observational study consisting of a 2-day visit at UNN for examinations according to a study protocol. Clinical participants without mask-based therapies for respiration disorders were additionally invited to a polysomnography (PSG) recording. The present study includes data from the two survey studies, the clinical study, and the PSG study (Fig. 1).

Procedure

The surveys were administered by regular mail. The sleep survey (consisting of instruments 1–4 described below) was

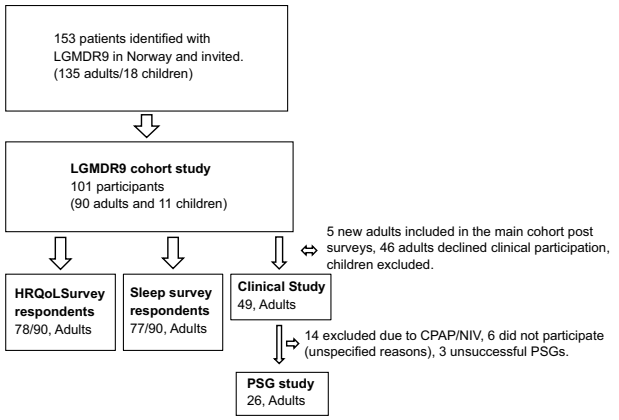

Fig. 1 Flow chart. *PSG* polysomnography, *CPAP* continuous positive airway pressure, *NIV* non-invasive ventilation

Table 1 The schedule for the clinical participants

Day 0	Check in at the patient hotel
Day 1	Capillary blood gas (7.30 a.m.)
	Weight/height
	Self-report instruments
	Electrocardiography
	Muscle ultrasound
	Motor tests
	Mounting of PSG (15 p.m.)
Day 2	Detachment of PSG (7.30 a.m.)
	Pulmonary lab
	Echocardiography
	Clinical neurological examination
	Cognitive test
	Motor tests

PSG polysomnography

administered 4 weeks after the HRQoL survey (consisting of instruments 5 and 6 below). The Fatigue Severity Scale and the respiratory questionnaire were also completed during the hospital visit. The schedule for the clinical study included capillary blood gas, clinical neurological examination, Body Mass Index (BMI), echocardiography with semi-automated estimation of the biplane left ventricular ejection fraction (EF), the 32-item Motor Function Measure, and pulmonary function tests (Table 1). For eligible participants, PSG with capnometry was performed within the frame of the clinical study. PSG equipment was attached in the afternoon by a technician at the Department of Clinical Neurophysiology, and the recording was conducted ambulatory at the patient hotel. SDB included sleep-related hypoventilation, sleep-related hypoxemia, and sleep apnea (SA) as defined below. EF was reported as EF < 50% (impaired) or ≥ 50% (normal). Data from the clinical study were collected and managed using REDCap[1] electronic data capture tools hosted at UNN [16, 17].

Self-report instruments

1) Fatigue: The Norwegian version of Fatigue Severity Scale (FSS) comprises nine items which are rated on a seven-point Likert scale 1–7 [18]. A mean item score ≥ 5 indicates clinically significant fatigue, based on recommendations in a Norwegian validation study [19]. Missing values were not replaced.
2) Excessive daytime sleepiness (EDS): The Norwegian version of Epworth Sleepiness Scale (ESS) includes eight items with a 0–3 response range representing low to high chance of dozing off in a given situation [20, 21]. A sum score > 10 indicates significant EDS. Missing values were not replaced.
3) Insomnia: The Bergen Insomnia Scale (BIS) was originally developed complying to the criteria for chronic insomnia of the fourth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-4) [22], and subsequently adjusted to the criteria of DSM-5/the third edition of the International Classification of Sleep Disorders (ICSD-3) [23]. The BIS contains six items (score range 0–7) indicating how frequently, i.e., days a week, the patient has experienced nighttime (three items) and daytime (two items) insomnia symptoms, and non-restorative sleep (one item). The time frame is the last three months. The minimum criterion for insomnia is a score of ≥ 3 on both one nighttime and one daytime item. The sum score of all six items (range 0-no to 42-maximum) indexes the level of symptom burden as a continuous score. Missing values were not replaced.
4) Sleep-related problems: Quality-of-life questions for patients on home mechanical ventilation (HMV) (here: «respiratory questionnaire») originate from a Swedish stress research program [24] and subsequent research on HRQoL in patients with chronic alveolar hypoventilation [25, 26] and implementation in the Swedish [27] and the Norwegian national register for patients on HMV [28]. It contains five items, and a previous study with NIV intervention showed that all items except daytime tiredness correlated with morning PaCO2 levels, which also related to generic HRQoL [26]. The translated Norwegian version used in the present study was not vali-

[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.

dated, and the severity scale in terms of frequency differed slightly from the Swedish version: never or almost never/sometimes/several times a week (Swedish version: once a week)/daily or almost daily. Missing values were not replaced.

5) HRQoL: The Norwegian version of the 36-item Short Form Health Survey (SF-36) version 1 [29] is a generic measure. The items are scored on a 2 to 6-point categorical scale aggregated into nine 0–100-point (100 = maximal HRQoL) subscales: physical functioning, role limitations due to physical problems, bodily pain, general health, vitality, social functioning, role limitations due to emotions, mental health, and change in health over the past year. Missing values were replaced according to the scoring algorithm.
6) HRQoL: The Norwegian version of the Individualized Neuromuscular Quality of Life questionnaire (INQoL) version 2.0 [30] is a disease-specific measure comprising seven symptom domains (i.e., muscle weakness, myalgia, fatigue, myotonia, diplopia, ptosis, and dysphagia), five life domains (i.e., activities related to daily living/leisure/work, independence, social relationships, emotions, and body image), and two treatment domains. The items are scored on a 7-point scale (Likert type, but each number also has a categorical description) transformed into separate domain scores, and an aggregated score (the INQoL index) as a proxy of disease burden or impact on HRQoL. The INQoL index is based only on the items on impact and impact importance of each life domain and does not include the items of the life domains that describe the perceived status. Each transformed score range 0–100 (100 = maximal burden). Missing values were replaced according to the scoring algorithm.

Pulmonary function tests (PFT)

Spirometry was performed according to The American Thoracic Society and the European Respiratory Society technical guidelines 2019 [31]. In addition to the standard sitting position, spirometry was also performed in the supine position. This has been recommended for patients with NMD since diaphragmatic dysfunction tends to cause a relatively low performance in the supine position [32]. We calculated the relative drop in Forced Vital Capacity (FVC) from sitting to supine position (ΔFVC), and FVC percent predicted in both positions (FVC% and FVC% supine), using reference equations for standard position from The Global Lung Function Initiative (GLI) 2021 [33]. Maximal inspiratory pressure (MIP) was performed in a sitting position and measured three times, but additional one or two times when the technique was inadequate. The best of repeated measurements was used and recorded as percent predicted (MIP%) using reference equations from GLI [34].

The 32-item motor function measure (MFM32)

MFM32 is a clinician-reported quantitative scale of motor function in individuals with NMD [35]. The scale is adapted to all degrees of severity; for walking and non-walking patients. Each item is scored on a 4-point Likert scale from 0 (cannot initiate the task) to 3 (performs the task fully). Total score ranges from 0 to 96 points, based on three subdomain scores: D1—Standing and transfers (39), D2—Axial and proximal motor function (36), and D3—Distal motor function (21). The result is expressed as percentage of the maximum possible score. The assessors were MFM32-certified physiotherapists with special expertise in neuromuscular disease.

PSG study

SOMNOscreen equipment and Domino version 2.7.0 software (Somnomedics, Randersacker, Germany) were used for PSG. PSG recording was performed in accordance with the guidelines of The American Academy of Sleep Medicine (AASM) version 2.4 (2017) [36]. Six electroencephalographic leads (F3/F4, C3/C4, O1/O2), right and left electrooculography, and submental electromyography were used for sleep scoring. Pressure flow oral-nasal cannula, inductive thoracic and abdominal belts, and oximetry (Nonin) were used for respiratory assessment. Body position was monitored by an accelerometer incorporated in the PSG device attached to the chest. PSG scoring was divided between two consultant clinical neurophysiologists at UNN (co-authors) and conducted according to AASM [36]. Total sleep time (TST), sleep efficiency, amount of REM sleep and deep (N3) sleep (minutes and %TST), time spent in supine position (%TST), and mean oxygen saturation (SpO_2) were reported and included in the analyses. Desaturation was defined as a ≥ 3% decrease in SpO_2. Apnea was scored when there was ≥ 10 s duration of ≥ 90% air flow reduction, and classified as obstructive, central, and mixed [36]. Hypopnea was scored when there was ≥ 10 s duration of ≥ 30% air flow reduction associated with a ≥ 3% decrease in SpO2 and/or an EEG arousal [36] and were not classified considering both the general risk of misclassification of hypopneas [37] and the additional risk in NMD where events related to respiratory muscle weakness may resemble or compound obstructive or central events [12, 14]. Oxygen desaturation index (ODI), apnea–hypopnea index (AHI), apnea index (AI), obstructive AI (oAI), central AI (cAI), and microarousal index (MAI) were calculated as events per hour of sleep. AHI was additionally calculated differentially in Non-REM (NREM) and REM sleep, and in supine and non-supine

position, and the respective ratios were reported among candidates with SA. We defined SA as AHI ≥ 5 independent of symptoms and comorbidities. SA severity was defined as mild (AHI 5.0–14.9), moderate (AHI 15.0–29.9), or severe (AHI ≥ 30.0) [36]. Sleep-related hypoxemia was defined as SpO2 ≤ 88% for > 5 consecutive minutes in accordance with ICSD-3 (AASM 2014) [6], but we used the definition regardless of concurrent capnometry outcomes. Additionally, we assessed time spent with SpO2 < 90% (minutes and %TST) and the presence of Cheyne-Stokes respiration.

SenTec Digital Monitoring System (SenTec AG, Therwil, Switzerland) was used for nocturnal transcutaneous PCO2 (PtcCO2) monitoring. The sensor was placed on the forehead. Current AASM criteria for sleep-related hypoventilation were applied: an increase in PtcCO2 to a value > 7.33 kPa (55 mmHg) for ≥ 10 min and/or ≥ 1.33 kPa (10 mmHg) increase in PtcCO2 during sleep in comparison to awake supine values to a value exceeding 6.67 kPa (50 mmHg) for ≥ 10 min [38]. Mean and maximal PtcCO2, and maximal rise in PtcCO2 from initial values were used as variables.

Statistical analyses

Data were analyzed using IBM SPSS Statistics for Windows (Version 28.0. Armonk, NY: IBM Corp.). Distribution of continuous data are described using median and interquartile range (IQR). Categorical variables are presented with frequencies. Simple group comparisons from continuous variables were performed with independent t-test with bootstrapping (5000 resamples). Group comparison from categorical variables were assessed with Pearson chi-square or Fischer exact test with mid p-correction, as appropriate, and significant findings presented with odds ratio (OR) with confidence interval (CI). Correlations were examined with Spearman rank correlation (r_s) or Pearson correlation (r) according to the distributions and inspected with scatter plot and curve estimation. In the assessment of predictors of AHI, multiple linear regression (with backward elimination method) was used due to several relevant relationships with potential confounding effects: age, sex, and BMI as well-established risk factors for SA [39] and among LGMDR9-related variables (pulmonary function indices, EF < 50%, macroglossia, and dysarthria or dysphagia), EF < 50% was found relevant based on p value in t test ($p < 0.10$). Assumption of normal distribution of the residuals was assessed with P–P plot, skewness, and kurtosis, homoscedasticity by scatter plotting predicted versus residual values, and influential cases in terms of Cook's distance and DeltaFit. The alpha level was set to $p < 0.05$. Considering the exploratory nature of the study, correction for multiple testing was not used in this study [40].

Results

Participants

Figure 1 provides an overview of the recruitment and inclusion of the participants. In total, 77/90 (86%) adults participated in the sleep survey, and the response rate of the four instruments ranged 72–75/90 (80–83%). The response rate of the HRQoL survey was 78/90 (87%). The inclusion rate for the clinical study was 49/95 (52%). Comparable levels of age, sex distribution, wheelchair dependency, and established mask-based therapies indicated that the samples participating in the two surveys and the clinical study were representative of the cohort. In the clinical study, 35/49 (14/22 females and 21/27 males) did not receive mask-based therapy and were thus eligible for PSG. However, six participants (five females and one male) either declined the invitation to PSG or were not invited due to practical inconvenience, and three recordings (of one female and two males) were unsuccessful, hence excluded. The 26 successful PSG registrations represented 8 of 14 (57%) eligible females and 18 of 21 (86%) eligible males.

Sleep and HRQoL surveys

Background data and outcomes of the sleep survey are presented in Table 2. There was a comparable number of female and male participants. The subgroup with mask-based therapies comprised eight respondents with continuous positive airway pressure support (CPAP) (i.e., 10% of all respondents) and 16 with NIV (i.e., 21% of all respondents) as bi-level positive airway pressure or ventilator, of which five also used NIV at daytime. The same subgroup had a female preponderance (63% vs 38%), was older (median age 56 vs 37 years), and had a higher prevalence of wheelchair dependency (67% vs 17%).

In total, 32% had insomnia and 10% EDS. Overlap of insomnia and EDS occurred in two respondents (Fig. 2). The prevalence of fatigue or frequent daytime tiredness ranged 37–40%. Frequent nightly awakenings and/or non-restorative sleep were common (36–49%), whereas 10% reported frequent morning headache and 3% frequent nocturnal dyspnea.

Insomnia was equally prevalent in the subgroup with nocturnal masks as in those without. Only one patient with mask-based therapy had EDS, i.e., severe residual sleepiness. Compared to the subgroup without masks, those who used masks showed a tendency towards a higher prevalence of nightly awakenings (55% vs 38%) and wake after sleep onset (> 30 min) (36% vs 12%) but a relatively lower

Table 2 Background data and outcomes of the sleep survey in participants without vs with mask-based therapies

	All n = 72–77 n (%) or M (IQR)	No mask n = 50–53 n (%) or M (IQR)	Mask n = 21–24 n (%) or M (IQR)
Females	37 (48)	22 (42)	15 (62.5)
Males	40 (52)	31 (58)	9 (37.5)
Age (years)	47 (32–58)	37 (27–54)	56 (50–63)
Daily smokers	4 (5)	3/53 (6)	1 (4)
COPD	1 (1)	1/53 (2)	0 (0)
W/C	25 (33)	9/52 (17)	16 (67)
ESS (0–24)	4.0 (2.0–8.0)	5.0 (2.0–8.0)	3.5 (0.0–8.0)
EDS (ESS > 10)	7 (10)	6 (12)	1 (5)
BIS (0–42)	9 (4–16)	9 (5–17)	8 (4–15)
Insomnia	23 (32)	16 (31)	7 (32)
SOL[a]	20 (27)	16 (31)	4 (18)
WASO[a]	14 (19)	6 (12)	8 (36)
Early awakening[a]	16 (22)	11 (22)	5 (23)
Non-restorative sleep[a]	35 (49)	27 (53)	8 (38)
Dissatisfaction w/sleep[a]	16 (22)	11 (22)	5 (23)
Daytime tiredness[a]	27 (37)	19 (37)	8 (36)
Respiratory questionnaire			
Several awakenings[b]	31 (43)	19 (38)	12 (55)
Morning headache[b]	7 (10)	5 (10)	2 (9)
Refreshing sleep[b]	46 (64)	32 (64)	14 (64)
Daytime tiredness[b]	29 (40)	21 (42)	8 (36)
Nocturnal dyspnea[b]	2 (3)	2 (4)	0 (0)
FSS (1–7)	4.3 (3.6–5.7)	4.2 (3.3–5.5)	4.7 (3.9–6.2)
Fatigue (FSS ≥ 5)	29 (40)	18 (36)	11 (48)

M median, *IQR* inter-quartile range, *COPD* chronic obstructive pulmonary disease, *W/C* wheelchair dependency, *ESS* Epworth Sleepiness Scale, *EDS* excessive daytime sleepiness, *BIS* Bergen Insomnia Scale, *SOL* sleep onset latency (> 30 min), *WASO* wake after sleep onset (> 30 min), *FSS* Fatigue Severity Scale

[a] ≥ 3 days a week for the last 3 months

[b] Several times a week or more

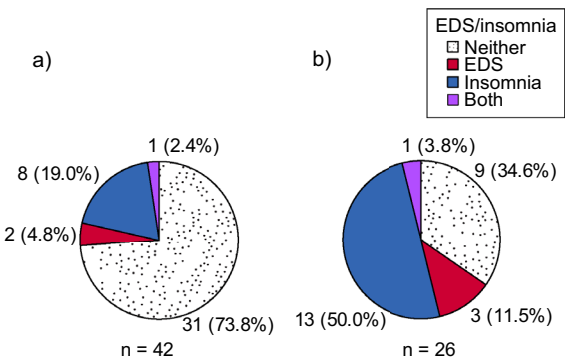

Fig. 2 Prevalence of insomnia and excessive daytime sleepiness (EDS) among LGMDR9 participants **a** without fatigue (Fatigue Severity Scale (FSS) < 5) and **b** with fatigue (FSS ≥ 5)

prevalence of sleep onset latency (18% vs 31%). Prevalence of early awakening and dissatisfaction with sleep in the two groups was comparable (22%).

The prevalence of insomnia was 36% in females and 27% in males (p = 0.40) and unrelated to wheelchair dependency (p = 0.81). Level of insomnia symptoms was not correlated with age ($r_s = 0.07$) or physical or social aspects of HRQoL but to poorer outcomes on mental aspects of HRQoL, especially vitality, pain, and fatigue (Table 3). Correspondingly, the prevalence of insomnia was increased in fatigued compared to non-fatigued patients; 54% vs 21%; (OR 4.41, CI 1.52–12.79, p = 0.005) (Fig. 2). Concurrently, the prevalence of EDS was relatively low (10%) and not significantly increased in fatigued patients (p = 0.30) (Fig. 2).

Table 3 Correlations between levels of insomnia (the sum score of Bergen Insomnia Scale) and health-related quality of life (HRQoL) in LGMDR9

HRQOL Subscales	r_s (n = 68–70)	95% CI	p
SF-36 physical	0.19	− 0.06, 0.41	0.13
SF-36 role physical	− 0.20	− 0.42, − 0.05	0.11
SF-36 bodily pain	− 0.41	− 0.60, − 0.19	0.0004
SF-36 general health	− 0.22	− 0.44, 0.03	0.07
SF-36 vitality	− 0.46	− 0.64, − 0.25	< 0.0001
SF-36 social	− 0.18	− 0.40, 0.07	0.14
SF-36 role emotional	− 0.35	− 0.55, − 0.11	0.003
SF-36 mental	− 0.38	− 0.57, − 0.15	0.0012
INQoL muscle weakness	− 0.03	− 0.27, 0.22	0.48
INQoL fatigue	0.40	0.17, 0.58	0.0007
INQoL myalgia	0.30	0.06, 0.50	0.01
INQoL activities	0.02	− 0.22, 0.26	0.86
INQoL independence	− 0.03	− 0.27, 0.21	0.80
INQoL relationships	0.006	− 0.24, 0.25	0.96
INQoL emotions	0.26	0.02–0.47	0.03
INQoL index	0.09	− 0.16, 0.32	0.48

Negative relationships with SF-36 and positive with INQoL mean that increasing levels of insomnia are associated with poorer HRQoL outcomes. Only subscales considered relevant in relation to sleep were included

r_s Spearman's rho, *CI* confidence interval, *SF-36* 36-item Short Form Health Survey, *INQoL* Individualized Neuromuscular Quality of Life questionnaire

PSG study and clinical study

Background data and outcomes of the PSG study and additional assessments of the 26 PSG candidates during their hospital visit are shown in Table 4. Females and males had comparable age and BMI, but females showed relatively poorer outcomes on pulmonary function tests and a higher proportion of males had an impaired EF. Two subjects were wheelchair dependent. The PSG candidates with indications of SDB, except mild SA, are presented individually in Table 5. One of the PSG candidates, a male with comorbid chronic obstructive pulmonary disease and an EF < 50% had tried mask-based therapy previously but not tolerated it (Table 5, Subject 1). PSG showed severe SA, and he was the only PSG candidate with Cheyne-Stokes respiration and a SpO2 < 90% exceeding 5% TST. He also met the criteria for insomnia in the survey, which may have contributed to the lack of tolerance to the mask. Nineteen PSG candidates had a successful capnometry. Mean PtcCO2 levels were median 5.69 kPa in females and 5.99 kPa in males and the highest mean value recorded was 6.62 kPa. Maximum PtcCO2 levels were median 6.13 kPa in females and 6.58 kPa in males and the highest level recorded was 7.25 kPa (Table 4). Only one met the applied criteria for sleep-related hypoventilation

Table 4 Background data and outcomes of the polysomnography (PSG) study and additional assessments of the PSG candidates during their hospital visit

	Females (n = 8) n (%) or median	Males (n = 18) n (%) or median	All (n = 26) Range
Characteristics			
Age (years)	40	41	16–64
BMI (kg/m^2)	26.2	26.5	21.0–36.4
BMI > 30 kg/m^2	1 (0)	3 (17)	–
Daily smokers	1 (13)	1 (6) N.D.: 1	–
W/C	1 (13)	1 (6)	–
Self-report instruments			
FSS (1–7)	4.4	4.1	2–6
Fatigue (FSS ≥ 5)	3 (38)	6 (33)	–
Respiratory questionnaire			
Several awakenings[a]	2 (25)	7 (41)	–
Morning headache[a]	1 (13)	1 (6)	–
Refreshing sleep[a]	4 (50)	10 (56)	–
Daytime tiredness[a]	6 (75)	8 (44)	–
Nocturnal dyspnea[a]	0 (0)	0 (0)	–
Cardiac/pulmonary function			
FVC%	72	89	60–111
FVC% sup	63 N.D.:1	85 N.D.:4	32–103
ΔFVC (%)	22 N.D.:1	11 N.D.:4	4–33
MIP%	62	81 N.D.:1	28–108
EF < 50%	1 (13)	7 (41) N.D.: 1	–
PSG study			
TST (hours:min)	6:31	6:44	4:50–8:57
Sleep efficiency (%)	92.0	90.2	63.0–97.6
Supine (%TST)	65.1	45.6	3.3–100.0
REM sleep (hours:min)	1:05	1:10	0:01–1:10
REM sleep (%TST)	15.6	17.0	0.0–28.5
N3 sleep (hours:min)	1:30	1:18	0:0–2:31
N3 sleep (%TST)	22.6	21.2	0.0–36.9
ODI (n/hour)	2.3	7.6	0.0–50.1
AHI (n/hour)	6.6	10.2	0.2–63.6
AI (n/hour)	1.8	3.6	0–42.4
oAI (n/hour)	1.3	1.5	0–34.7
cAI (n/hour)	0.0	0.3	0–7.3
AHI 5.0–14.9/hour	2 (25)	6 (33)	–
AHI 15.0–29.9/hour	2 (25)	4 (22)	–
AHI ≥ 30.0/hour	0 (0)	2 (11)	–
MAI (n/hour)	22.3	54.1	6.8–91.9
Mean SpO2 (%)	97	96	93–98

Table 4 (continued)

	Females (n = 8) n (%) or median	Males (n = 18) n (%) or median	All (n = 26) Range
Mean PtcCO2 (kPa)	5.69 N.D.: 3	5.99 N.D.: 4	5.23–6.62
Max PtcCO2 (kPa)	6.13 N.D.: 3	6.58 N.D.: 4	5.48–7.25

BMI body mass index, *N.D.* no data, *W/C* wheelchair dependency, *FSS* Fatigue Severity Scale, *FVC%* forced vital capacity percent predicted, *FVC%* $_{sup}$ FVC in supine position percent of predicted FVC in standard position, Δ*FVC* relative FVC drop from sitting to supine position, *MIP%* maximal inspired pressure percent predicted, *EF* left ventricular ejection fraction, *TST* total sleep time, *REM* rapid eye movement, *N3* non-REM sleep stage 3, *ODI* oxygen desaturation index, *AHI* apnea–hypopnea index, *MAI* microarousal index, *SpO2* oxygen saturation, *PtcCO2* transcutaneous carbon dioxide tension

[a]Several times a week or more

(Table 5, Subject 9). No correlation between PtcCO2 levels and PFT for any of the variables included in the study (see method section) was found. None met the applied criterion for sleep-related hypoxemia. SA was detected in 16/26 PSG candidates (4/8 females and 12/18 males) and was moderate or severe in 8/26 (Table 4). Among the 16 subjects with SA, median oAI was 6.8/h and cAI 0.2/h and the oAI comprised > 50% of AHI in one half, whereas the hypopneas predominated in the other half. A cAI ≥ 5 was only recorded in one subject. This subject had an implanted cardiac resynchronization therapy defibrillator and an EF < 50% but also a predominant, severe OSA (Table 5, Subject 2).

All four PSG candidates with a BMI > 30 kg/m^2 and all eight with EF < 50% had SA. AHI was relatively increased

Table 5 Characteristics and measurements of the polysomnography candidates with hypoventilation or moderate/severe sleep apnea

Subject no	1	2	3	4	5	6	7	8	9
Age (years)	62	55	46	51	59	57	44	49	31
Sex	M	M	M	F	M	F	M	M	M
BMI (kg/m^2)	27.2	25.6	22.9	23.4	26.8	24.5	28.5	23.8	21.9
W/C	+	−	−	−	−	+	−	−	−
COPD	+	−	−	−	−	−	−	−	−
EF < 50%	+	+[a]	−	+	−	−	+	−	− (50%)
Respiratory metrics									
SA	+	+	+	+	+	+	+	+	−
AHI n/hour	43.3	63.6	21.6	21.4	20.7	20.6	16.3	16.9	1.4
AI n/hour	3.9	42.4	9.4	3.8	18.0	11.0	11.4	13.7	1.1
oAI n/hour	0.3	34.7	9.4	3.8	18.0	10.9	8.8	12.4	1.1
cAI n/hour	0.3	7.3	0.0	0.0	0.0	0.2	1.7	1.2	0.0
ODI n/hour	41.2	50.1	18.2	3.7	10.2	4.0	7.8	7.7	0.5
CSR	+	−	−	−	−	−	−	−	−
SpO2 < 90% min (%TST)	72 (16)	15 (4)	6 (1)	3 (< 1)	3 (1)	1 (< 1)	4 (1)	1 (1)	3 (< 1)
HV	−	−	−	−	N.D	−	−	−	+
FVC%	42	85	107	88	88	75	100	115	104
FVC%$_{sup}$	32	N.D	99	63	N.D	73	87	103	88
ΔFVC (%)	25	N.D	8	28	N.D	4	13	10	16
MIP%	54	N.D	107	59	94	81	92	81	65
Symptoms									
Several awakenings[b]	+	+	−	−	−	−	+	−	−
Morning headache[b]	+	−	−	−	−	−	−	−	−
Refreshing sleep[b]	−	−	+	+	+	+	+	+	+
Daytime tiredness[b]	−	+	−	+	+	+	−	−	+
Nocturnal dyspnea[b]	−	−	−	−	−	−	−	−	−
FSS (1–7)	4.1	5.9	1.9	4.4	4.1	3.7	1.8	2.3	4.2

M male, *F* female, *BMI* body mass index, *W/C* wheelchair dependency, *COPD* chronic obstructive pulmonary disease, *EF* ejection fraction, *SA* sleep apnea (AHI ≥ 5), *AHI* apnea–hypopnea index, *AI* apnea index, *oAI* obstructive AI, *cAI* central AI, *ODI* oxygen-desaturation index, *CSR* Cheyne-Stokes respiration, *SpO2* oxygen saturation, *TST* total sleep time, *HV* sleep-related hypoventilation, *N.D.* no data, *FVC%* forced vital capacity percent predicted, *FVC%* $_{sup}$ FVC in supine position percent of predicted FVC in standard position, Δ*FVC* relative FVC drop from sitting to supine position, *MIP%* maximal inspired pressure percent predicted, *FSS* Fatigue Severity Scale

[a]Implanted cardiac resynchronization therapy defibrillator

[b]Several times a week or more

Table 6 Multivariate regression model with stepwise backward elimination of non-significant predictors. The apnea–hypopnea index (AHI) from the polysomnography candidates as dependent variable

Dependent variable: AHI (n = 24)						
Model	R^2/R^2_{adj}	Predictor	Beta	β	p[a]	95% CI for beta
1	0.544/0.453	EF < 50% (vs ≥ 50%)	13.25	0.43	0.019 (0.011)	2.46, 24.03
		BMI (kg/m^2)	− 0.56	− 0.14	0.399 (0.820)	− 1.90, − 0.79
		Age (years)	0.59	0.56	0.002 (< 0.001)	0.24, 0.94
		Male (vs female)	− 1.36	− 0.04	0.789 (0.931)	− 11.77, 9.06
2	0.543/0.477	EF < 50% (vs ≥ 50%)	12.79	0.42	0.014 (0.007)	2.84, 22.74
		BMI (kg/m^2)	− 0.51	− 0.13	0.411 (0.828)	− 1.78, 0.76
		Age (years)	0.58	0.55	0.002 (< 0.001)	0.25, 0.92
3	0.527/0.485	EF < 50% (vs ≥ 50%)	11.75	0.39	0.018 (0.005)	2.23, 21.26
		Age (years)	0.56	0.54	0.002 (< 0.001)	0.24, 0.90

Normality assumptions for the residuals were not met. Square root transformation of the AHI variable normalized the residuals and yielded similar findings as the untransformed model

R^2 explained variance, *adj* adjusted, *beta* unstandardized regression coefficient, *β* standardized beta, *CI* confidence interval, *EF* ejection fraction, *BMI* body mass index

[a]p-value based on transformed data

in those with an EF < 50% compared to those with a normal EF (p = 0.096). AHI was uncorrelated with the PFT metrics (r_s = 0.18 to − 0.06, p ≥ 0.40) and unrelated to macroglossia (n = 4) and dysarthria and/or dysphagia (n = 3). Multiple regression showed that AHI correlated with advancing age and an EF < 50% but not with BMI or sex (Table 6). We assessed the relationships between AHI and sleeping position and REM sleep, respectively. Respiratory events tend to increase in the supine sleeping position and patients with LGMDR9 may be more prone to the supine position due to mobility difficulties. REM sleep tend to aggravate OSA and events related to respiratory muscle weakness due to physiological REM atonia, as mentioned, whereas central apneas usually occur in NREM sleep [41]. Among the subjects with SA, median AHI ratio supine/non-supine was 3.2 (range 1.3–16.4) and median AHI ratio REM/NREM was 2.7 (range 0–6.2).

The AHI was unrelated to the items of the respiratory questionnaire. However, the two individuals with severe SA stood out with problems on three of five items (Table 5, Subject 1 and 2). No correlations between FSS and nocturnal measurements were detected including TST, amount N3 sleep (minutes and %TST), amount REM sleep (minutes and %TST), AHI, MAI, ODI, mean SpO2, and mean PtcCO2. Nevertheless, relationships between FSS and pulmonary function impairment were observed and most clearly between FSS and MIP% (Table 7, Fig. 3). In the subgroup with SDB, a correlation also between FSS and ΔFVC was found. In the whole group without mask-based therapies, FSS correlated both with MIP% and FVC % supine but not

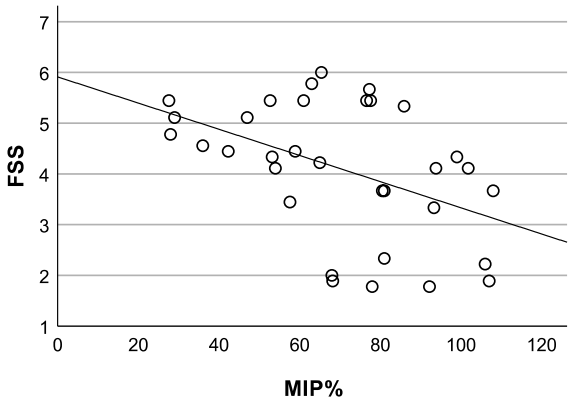

Fig. 3 Scatter plot showing the relationship between Maximal Inspiratory Pressure percent predicted (MIP%) and Fatigue Severity Scale (FSS) (range 1–7, i.e., no to maximal fatigue) in the clinical participants without mask-based therapies

with MFM32 (D3: r = − 0.25, D2: r = − 0.20, D1: r = − 0.10, total score: r = − 0.15) and capillary PCO2, bicarbonate, or base excess. Comparatively, in the group with established mask-based therapies, no correlation between FSS and PFT metrics was detected and there was even a tendency of an inverse relationship between fatigue severity and ΔFVC (Table 7). This group consisted of four subjects with CPAP and ten subjects with NIV (one also partly daytime and another 24 hours) and had relatively higher levels of age, BMI, and wheelchair dependency as well as poorer outcomes on PFT compared to the group without mask-based therapy (Table 7).

Table 7 Characteristics, pulmonary function tests (PFT), and correlations between PFT and Fatigue Severity Scale (FSS) in clinical participants without vs with mask-based therapies

	No mask			Mask
	All no mask n = 35	PSG study n = 26	SDB[a] n = 17	n = 14
Age (years), median	38	41	47	56
BMI (kg/m^2), median	25.8	26.5	26.8	30.4
W/C, n (%)	6 (17)	2 (8)	2 (12)	10 (71)
FSS, median	4.3	4.2	4.1	4.6
FVC%, median	88	89	93	59 N.D.: 1
FVC% - FSS, r	− 0.27, p = 0.12	− 0.06, p = 0.78	− 0.07, p = 0.79	− 0.03, p = 0.93
FVC% sup, median	74 N.D.: 9	77 N.D.: 5	85 N.D.: 5	40 N.D.: 3
FVC% sup - FSS, r	− 0.39, p = 0.046	− 0.25, p = 0.27	− 0.35, p = 0.27	0.11, p = 0.74
ΔFVC (%), median	12 N.D.: 9	11 N.D.: 5	11 N.D.: 5	19 N.D.: 3
ΔFVC (%) - FSS, r	0.20, p = 0.34	0.37, p = 0.10	0.59, p = 0.043	− 0.53, p = 0.09
MIP% median	68 N.D.: 2	78 N.D.: 1	80 N.D.: 1	54 N.D.: 1
MIP% - FSS, r	− 0.46, p = 0.008	− 0.43, p = 0.030	− 0.54, p = 0.031	− 0.02, p = 0.94

r = Pearson correlation coefficient

PSG polysomnography, *SDB* sleep-disordered breathing, *BMI* body mass index, *W/C* wheelchair dependency, *FVC%* forced vital capacity percent predicted, *FVC%* supine FVC supine percent of predicted FVC in standard position, *N.D.* no data, *ΔFVC* relative FVC drop from sitting to supine position, *MIP%* maximal inspired pressure percent predicted

[a]PSG candidates where SDB was detected:16 with sleep apnea and one with hypoventilation according to the applied criteria

Discussion

In this study of a national cohort with LGMDR9, insomnia was prevalent in both subjects with and without mask-based therapies and significantly more prevalent among fatigued compared to non-fatigued patients. Insomnia severity correlated negatively with mental HRQoL. The PSG study uncovered a high occurrence of previously unrecognized SA, which was predominated by obstructive apneas in 50% and by unclassified hypopneas in the remaining and was related to the supine sleeping position and to REM sleep. Central apneas were relatively infrequent and only one had Cheyne-Stokes respiration. AHI was correlated with increasing age and an impaired EF. One subject met the AASM criteria for nocturnal hypoventilation. Fatigue severity was unrelated to nocturnal measurements of sleep and respiration, morning capillary blood gas, and motor function but negatively correlated with pulmonary function, particularly inspiratory muscle strength.

Insomnia

Insomnia was more prevalent relative to two previous Norwegian population studies (32% vs 20%) [23, 42]. Co-existing EDS and insomnia was rare, which resonates with previous knowledge that sleepiness is usually not present in insomnia [7]. Potential contributors to an increased prevalence of insomnia in LGMDR9 are immobility, myalgia, mental distress, and SA [8, 43–45]. This study showed that levels of insomnia were associated with mental aspects of HRQoL including pain. Previously, we found that both mental, physical, and social aspects of SF-36, but not pain, were significantly poorer in our LGMDR9 cohort compared to a reference population (Jensen SM et al., submitted paper). Insomnia was also related to the mental aspects SF-36 vitality and INQoL fatigue and the increased prevalence of insomnia in fatigued compared to non-fatigued patients substantiates this relationship. This finding aligns with the aforementioned study in slowly progressive muscular dystrophies [10] and with a study of a large clinical sample with insomnia [46]. In the latter study, a bidirectional relationship was proposed and depression was identified as a significant mediator. Worth noting is that CBT-I is also recommended for comorbid insomnia, particularly as it may potentially improve accompanying conditions such as pain or depression [7].

Patients who received mask-based therapy also had a high prevalence of insomnia, but the nocturnal pattern differed such that there were less difficulties with sleep initiation and more excessive wake time throughout the night, compared to patients without a mask. However, early morning awakening was comparable in both subgroups. Insomnia in these patients may reflect inappropriate mask-based therapy or the need for additional intervention for insomnia. As aforementioned, insomnia in patients with mask-based therapy is a particular concern since it may affect adherence and, resultantly, treatment outcomes. The

potential need for interdisciplinary treatment in comorbid SDB and insomnia may be a limiting factor.

Sleep-disordered breathing (SDB)

The diagnosis of SDB relies on objective measurements of respiratory functions during sleep. Symptoms of untreated or sub-optimally treated SDB may reflect in the respiratory questionnaires, gauging of morning headache and nocturnal dyspnea, and the ESS assessing EDS. Although EDS has heterogeneous causes, it is commonly associated with SDB [47]. The survey showed that the prevalence of these potential symptoms of SDB was low. EDS was even relatively low compared to a previous Norwegian general population study (9.6% vs 17.7%) [48].

AHI was elevated in a high proportion of our PSG candidates. This was not unexpected considering the high prevalence in general populations [39]. Furthermore, applying the specified criteria for sleep-related hypoventilation and hypoxemia, only one mild case of sleep-related hypoventilation was detected. Nevertheless, as previous studies have demonstrated, this highly depends on the diagnostic criteria [49–51]. In Duchenne muscular dystrophy, disease-specific treatment criteria for hypoventilation, more liberal than AASM criteria, have been established, yet comparative trials remain [9, 51]. However, a low rate of sleep-related hypoventilation in our study can be explained by the sample. Compared to those with established mask-based therapy, the PSG sample represented a relatively younger age group and earlier stages of disease by a low rate of wheelchair dependency and more preserved pulmonary function. Additionally, the group with mask-based therapy had a relatively higher rate of obesity (median BMI > 30 kg/m^2), which is an independent risk factor for hypoventilation (obesity-hypoventilation syndrome).

While the AHI in one half of those with SA was predominated by obstructive apneas, the other half had predominantly hypopneas, which could be obstructive, central, diaphragmatic, or compounded. AHI was related to advancing age, which is a general risk factor for OSA [39] but could also be related to progression of the disease. Concerning comorbid associations with SA, a Finnish nationwide registry-based case–control study found that SA (including OSA and CSA) is strongly associated with obesity, heart failure, and respiratory disease [52]. In concurrence, we found an association with heart failure. Previous studies have shown that heart failure is associated with both CSA and OSA and suggested that heart failure may promote OSA through CSA or upper airway edema [53], but the mechanisms have not been established. In the current study, central apneas were rare. Nonetheless, considering the unspecified nature of the hypopneas, this study cannot distinguish whether the correlation between AHI and an EF < 50% relates to a specific type of events. Further, the low BMI levels and relatively preserved pulmonary function among our PSG candidates, in general, may explain why BMI and pulmonary metrics did not turn out as significant correlates of the AHI. This may also indicate that SA is not underrecognized among patients with obesity or significant respiratory involvement since treated patients were not included in the PSG study. A report from a task force of the European Respiratory Society and the European Sleep Research Society advised to take the individual susceptibility into account in treatment decisions [54]. Comorbidities like heart failure, obesity, and respiratory muscle weakness may represent such vulnerabilities. Furthermore, SA treatment has shown potential to improve comorbidities such as obesity, metabolic disease, and heart failure [53, 55], which emphasizes the importance of considering morbidity profile in the monitoring of SDB.

AHI was related to the supine sleeping position and to REM sleep, which is well known in OSA [56] but could also be related to diaphragm weakness [14]. Since the sleeping position and the proportion of REM sleep during PSG registration may differ from habitual sleep without recording devices, these variables also need to be considered to avoid under or overdiagnosis. Additionally, the association with position means that positional therapy, preventing supine sleeping position, may be an option. Nevertheless, patients with muscle disease may need special consideration due to mobility or pain issues.

AHI was poorly correlated with sleep-related symptoms. However, research shows that SA is commonly sub-clinical, and both symptomatic and sub-clinical SA may be associated with cardiovascular disease [57]. This means that detection and intervention could be relevant independent of symptoms and consequently that regular sleep studies may be required in risk patients. Additionally, since symptoms and AHI are poor predictors of severity, there also exists a need for better biomarkers for treatment decisions [54].

Our study did not support the idea that fatigue in our LGMR9 cohort could be related to untreated SDB. However, we did find fatigue severity to be negatively correlated with inspiratory muscle strength (MIP). Since FSS was uncorrelated with metrics of blood gases and motor function, the mechanism of the FSS-MIP relationship seems more likely to be the work of breathing rather than an association with general motor function impairment or respiratory disturbances caused by the inspiratory weakness. The FSS-MIP relationship was absent in the group with established mask-based therapy. Potential explanations are the alleviation of breathing work due to ventilatory support and less physical exposure due to a higher rate of wheelchair dependency. Interestingly, a correlation between FSS and MIP has also been found in polio-myelitis [58] and several other studies have shown that MIP is a clinically meaningful outcome measure for NMD [59–61].

Strengths and limitations

This study provides insights into the prevalence of insomnia and unrecognized SDB in LGMDR9 and clinically relevant relationships with these sleep disorders and with fatigue. Important strengths of the surveys were sample sizes and response rates. The clinical study included a representative sample of the cohort and integrated assessments with standardized methods. PSG and PCO2 monitoring are considered gold standard methods for diagnosing SDB.

The study also has several limitations. Due to the exploratory design, we accepted a higher risk of conducting type I error (false positive), and instead of correcting for multiple testing, we urge for caution in assigning significance to p-values in the range of 0.01 to 0.05. Findings that are flagged as significant require replication. The prevalence of chronic insomnia disorder may be underestimated as the BIS does not cover all daytime impairments included in ICSD-3 (e.g., cognitive impairment, mood disturbance, and impaired motivation) or overestimated since a clinical interview is required to rule out exclusion criteria [7], such as insomnia due to poor sleep environment or insomnia mimics like circadian rhythm problems or restless legs syndrome. The PSG sample size was low, which increases the risk of type II errors (false negative conclusions), and females were relatively underrepresented. Identification of individuals with SDB relied on diagnostic criteria, which are mostly based on expert opinions [49, 60]. PSG may overestimate AHI by its tendency to increase the time in supine position [61] or underestimate it by reducing the proportion of REM sleep [62]. Sleep during PSG may have been impacted by an unfamiliar sleeping environment and setting and myalgia or tiredness after physical tests. However, TST, percentage of N3 sleep, and sleep efficiency indicated successful registrations. Technical issues limited the data completeness on capnometry and supine spirometry. Lastly, the study was conducted during the COVID-19 pandemic. Although the data were collected in relatively normal periods, this may have impacted the subjective measurements (HRQoL, sleep, and fatigue).

Conclusions

The study indicates that insomnia is prevalent in LGMDR9 and related to mental HRQoL. Correlations indicate the need for particular attention to insomnia in patients with fatigue, pain, or negative emotions since insomnia treatment may also relieve associated symptoms. The prevalence study also suggests a need for increased recognition of insomnia in patients receiving mask-based therapies as insomnia may affect device adherence and, consequently, treatment outcomes. While mask-based therapy was established in 31% of participants, SA was underrecognized among remaining participants, and identified risk factors were advancing age and an EF < 50%. Since SA treatment may benefit cardiac outcomes, sleep studies in patients with heart failure should be considered in particular. Fatigue was related to MIP but not motor function. This relationship advocates for pulmonary function tests in fatigued patients and suggests that MIP is a clinically meaningful measure in LGMDR9. More studies on respiratory natural history of LGMDR9 and biomarkers of SDB are required to decide proper indication and timing of pulmonary function tests, sleep studies, and treatment of SDB.

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Author contributions Study conception and design: SMJ and KAA. Collection data: SMJ (questionnaires, clinical neurological examination, and capnometry), KA (polysomnography), AR (echocardiography), CO (polysomnography), KIM (clinical neurological examination), ADR (the 32-item Motor Function Measure), MLV (pulmonary function tests), and KAA (clinical neurological examination). Performed the analyses: SMJ with support from OF and KA. SMJ wrote the initial draft and all critically revised it for important intellectual content and approved the final manuscript.

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Data availability The supporting data are not publicly available due to ethical restrictions. The participants have not given written consent for their data to be shared publicly.

Declarations

Conflicts of interest The authors declare that they have no conflict of interest.

Ethics approval The study was approved by the Regional Committee for Medical and Health Research Ethics of Northern Norway (2018/1968/REK nord), and by the Data Protection Officer at University Hospital of North Norway (UNN), and therefore performed in accordance with the Declaration of Helsinki and its later amendments. Agreements for use of the licensed forms (Fatigue Severity Scale,[2] the Epworth Sleepiness Scale,[3] and the Individualized Neuromuscular Quality of Life questionnaire[4]) were obtained. Permission to use

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the Bergen Insomnia Scale was obtained by correspondence with the Norwegian Competence Center for Sleep Disorders and the respiratory questionnaire by correspondence with the Norwegian Register for Long-Term Mechanical Ventilation and with B. Midgren. The Norwegian version of the 36-item Short Form Health Survey version 1 was freely distributed by The Knowledge Centre for the Health Services, Norwegian Institute of Public Health. The assessors of the 32-item Motor Function Measure were certified by the AFEHM association (Aide aux Familles d'Enfant Handicapé Moteur).

Informed consent All persons provided written informed consent for the collection and use of clinical data prior to their inclusion in the study.

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Appendix

Deltakers løpenr: ☐☐☐☐

Medisinsk genetisk nr: ☐☐☐☐

Deltakers initialer: ☐☐☐☐

to første bokstaver fra fornavn
+ to første fra siste etternavn

Fylles ut av studie medarbeider.

Limb-girdle muskeldystrofi 2I - spørreskjema til deltakere

Veiledning:
Deltakere under 16 år fyller ut sammen med foreldre/foresatte og hopper over Del 1 Sosialt.
Øvrige fyller ut alle deler.

Dato for utfylling ☐☐ . ☐☐ . ☐☐☐☐
dag måned år

GENERELT

Kjønn:

☐ Jente/kvinne ☐ Gutt/mann

Bostedsfylke: ☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐☐

Fødselsår/mnd: ☐☐☐☐ / ☐☐ (F.eks. 1980/10)

Del 1. SOSIALT

1. Er du gift/samboer?

☐ Ja ☐ Nei

2. Hva er din bosituasjon?

☐ Bor alene

☐ Bor med minst en annen voksen person

☐ Bor med kun barn (under 18 år)

3. Har du hjelpebehov i det daglige? (Evt. flere kryss)

☐ Nei, er selvhjulpen

☐ Ja, får noe hjelp fra familie/bekjente, ellers selvhjulpen

☐ Ja, har brukerstyrt personlig assistent

☐ Ja, har hjemmetjeneste

☐ Ja, bor i omsorgsbolig

☐ Ja, bor på institusjon

4. Hva er ditt høyeste utdanningsnivå?

☐ Grunnskole

☐ Videregående skole, yrkesfag

☐ Videregående skole, studieforberedende/allmennfag

☐ 1-3 år på høyskole/universitet

☐ 4 år eller mer på høyskole/universitet

5. Er du i arbeid/studerer?

☐ Ja, 100%

☐ Ja, men deltid pga nedsatt arbeidsevne

☐ Nei, 100% langtidsykemeldt/uføretrygdet

Fra hvilken alder? ☐☐ år

☐ Nei, men ikke pga. sykdom (Hjemmeværende, alderspensjonist, arbeidsledig, annet)

6. Dersom du har redusert/manglende arbeidsevne, skyldes dette i hovedsak LGMD 2I?

☐ Ja ☐ Nei

Del 2. MUSKELFUNKSJON

7. Hva var ditt første symptom på muskelsykdom? Sett ett eller flere kryss

☐ Forsinket motorisk utvikling

Den beste funksjonen blant nedenstående jeg har hatt: (1 kryss)

☐ Løpe ☐ Gå selvstendig ☐ Stå selvstendig ☐ Sitte uten støtte ☐ Sitte med støtte

☐ Hengte etter jevnaldrende ved løping/fysisk aktivitet

☐ Svakhet i ben/hofter

☐ Svakhet i armer/skuldre

☐ Svakhet i rygg

☐ Svakhet i magemuskler

☐ Slitenhet i kroppen

☐ Muskelsmerter/stivhet/kramper

☐ Hjertesymptomer

☐ Tungpust

☐ Ingen symptomer. Jeg tok gentest fordi det er andre i familien med sykdommen.

☐ Annet, beskriv:

8. Ved hvilken alder fikk du første symptom? ☐☐ år

9. Trenger du hjelpemidler for å forflytte deg?

☐ Nei, jeg går uten støtte

☐ Ja, trenger ganghjelpemidler (gåstol, krykker, stokk etc.)

Hvis behov for ganghjelpemidler, fra hvilken alder? ☐☐ år

☐ Jeg er avhengig av rullestol

Hvis avhengig av rullestol, fra hvilken alder? ☐☐ år

10. Dersom du ikke er avhengig av rullestol ved forflytning, hvilken gangdistanse klarer du på flat mark med eller uten ganghjelpemidler? (Sett 1 kryss)

☐ Ubegrenset

☐ Mer enn 1 km

☐ Mer enn 500 m

☐ Mer enn 50 m

☐ Under 50 m, men mer enn noen skritt

☐ Noen skritt

11. Dersom du har gangfunksjon, kan du løpe?

☐ Ja, uten problemer ☐ Ja, men ikke fort ☐ Nei

12. Dersom du har gangfunksjon, kan du gå opp trapper en etasje uten hjelp?

☐ Ja ☐ Ja, vha. gelender ☐ Nei

13. Bedriver du sport?

☐ Nei ☐ Ja

Beskriv:

14. Har du merket svakhet i armer/skuldre?

☐ Nei ☐ Ja

Hvis ja, fra hvilken alder? ☐☐ år

15. Har du fått påvist skjev ryggsøyle (skoliose)?

☐ Nei ☐ Ja

Hvis ja, ved hvilken alder? ☐☐ år

Hvis ja, er den behandlet? (evt. flere kryss)

☐ Nei, ingen behandling

☐ Ja, fysioterapi

☐ Ja, korsett

☐ Ja, operert

16. Har du vært plaget med pustevansker?

☐ Nei ☐ Ja

Hvis ja, fra hvilken alder? ☐☐ år

17. Har du behov for ventilasjonsstøtte (pustestøtte), for eksempel Bi-PAP?

☐ Nei

☐ Ja Fra hvilken alder? ☐☐ år

Når bruker du pustestøtte? ☐ Kun natt ☐ Natt og dag

Hvilken type pustestøtte?

☐ CPAP

☐ Bi-pap

☐ Annen Spesifiser:

18. Har du fått påvist hjertefeil/hjertesvikt relatert til LGMD 2I?

☐ Nei ☐ Ja

Hvis ja, ved hvilken alder? ☐☐ år

19. Har du hatt andre symptomer som kan relateres til LGMD2I (for eksempel utydelig tale, svelgvansker etc.)?

☐ Nei ☐ Ja

Hvis ja, beskriv symptomene og alder da disse startet:

20. Bruker du smertestillende medisiner mot muskelsmerter?

☐ Aldri

☐ Sporadisk

☐ Daglig/nesten daglig

Del 3. OPPFØLGING

21. Følges du opp hos barnelege/nevrolog?

☐ Ja ☐ Nei

22. Følges du opp hos lungelege?

☐ Ja ☐ Nei

Hvis Nei, har du tidligere fått utredning hos lungelege?

☐ Ja ☐ Nei

23. Følges du opp hos hjertelege?

☐ Ja ☐ Nei

Hvis Nei, har du fått utredning hos hjertelege?

☐ Ja ☐ Nei

24. Har du regelmessig fysioterapi?

☐ Ja ☐ Nei

Hvis Nei, hva er viktigste begrunnelse: (evt. flere kryss)

☐ Ikke behov

☐ Mangler tilbud i kommunen

☐ Ikke opplevd nytte av tilgjengelig behandling

☐ Behandlingen er kostbar

☐ Tungvint med transport

☐ Ønsker ikke fravær fra skole/jobb

☐ Annet, beskriv:

25. Har du hatt rehabiliteringsopphold?

☐ Ja, ett ☐ Ja, flere ☐ Nei

Hvis Nei, hva er grunnen til det? (evt. flere kryss)

☐ Ikke behov

☐ Ikke blitt forespurt

☐ Jeg har ikke ønsket det

☐ Begrenset tilbud

☐ Annet, beskriv:

26. Har du oppfølging i psykisk helsetjeneste pga belastninger relatert til LGMD 2I?

☐ Ja ☐ Nei, ikke behov ☐ Nei, men behov

Hvis Nei, men behov, hva er årsak til at du ikke har psykisk oppfølging?

☐ Ingen har forespurt

☐ Manglende tilbud

☐ Annet, beskriv:

Del 4. ANDRE MEDISINSKE TILSTANDER OG MEDIKAMENTER

27. Har du andre tilstander/sykdommer som medfører fysiske begrensninger?

☐ Nei ☐ Ja

Beskriv:

28. Har du hjerteproblemer som IKKE er relatert til LGMD 2I?

☐ Nei ☐ Ja

Beskriv:

29. Har du lungesykdom som IKKE er relatert til LGMD 2I
(for eksempel astma/kronisk obstruktiv lungesykdom (KOLS))?

☐ Nei ☐ Ja

Beskriv:

30. Har du andre medisinske tilstander som svekker din helse/ livskvalitet?

☐ Nei ☐ Ja

Beskriv:

31. Røyker du?

☐ Aldri røkt

☐ Nei, men røkte tidligere av og til

☐ Nei, men røkte tidligere daglig

I hvor mange år? | | | år

Hvilket år sluttet du? | | | | |

☐ Ja, av og til

☐ Ja, daglig

32. Bruker du faste medisiner?

☐ Nei ☐ Ja

Følgende medisiner (dosering/styrke ikke nødvendig å oppgi):

33. Har du tidligere benyttet kolesterolmedisiner?

☐ Nei ☐ Ja

Takk for din utfylling.