RESEARCH ARTICLE

Long-term Natural History of Pediatric Dominant and Recessive *RYR1*-Related Myopathy

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Abstract

Background and Objectives

RYR1-related myopathies are the most common congenital myopathies, but long-term natural history data are still scarce. We aim to describe the natural history of dominant and recessive *RYR1*-related myopathies.

Methods

A cross-sectional and longitudinal retrospective data analysis of pediatric cases with *RYR1*-related myopathies seen between 1992-2019 in 2 large UK centers. Patients were identified, and data were collected from individual medical records.

Results

Sixty-nine patients were included in the study, 63 in both cross-sectional and longitudinal studies and 6 in the cross-sectional analysis only. Onset ranged from birth to 7 years. Twenty-nine patients had an autosomal dominant *RYR1*-related myopathy, 31 recessive, 6 de novo dominant, and 3 uncertain inheritance. Median age at the first and last appointment was 4.0 and 10.8 years, respectively. Fifteen% of patients older than 2 years never walked (5 recessive, 4 de novo dominant, and 1 dominant patient) and 7% lost ambulation during follow-up. Scoliosis and spinal rigidity were present in 30% and 17% of patients, respectively. Respiratory involvement was observed in 22% of patients, and 12% needed ventilatory support from a median age of 7 years. Feeding difficulties were present in 30% of patients, and 57% of those needed gastrostomy or tube feeding. There were no anesthetic-induced malignant hyperthermia episodes reported in this cohort. We observed a higher prevalence of prenatal/neonatal features in recessive patients, in particular hypotonia and respiratory difficulties. Clinical presentation, respiratory outcomes, and feeding outcomes were consistently more severe at presentation and in the recessive group. Conversely, longitudinal analysis suggested a less progressive course for motor and respiratory function in recessive patients. Annual change in forced vital capacity was −0.2%/year in recessive vs −1.4%/year in dominant patients.

Discussion

This clinical study provides long-term data on disease progression in *RYR1*-related myopathies that may inform management and provide essential milestones for future therapeutic interventions.

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Glossary

CCD = central core disease; **CM** = congenital myopathies; **DDH** = dysplasia of the hip; **DNC** = Dubowitz Neuromuscular Centre; **FVC** = forced vital capacity; **HSS** = highly specialized service; **IDS-S** = infant disease severity rating scale; **MHS** = malignant hyperthermia susceptibility; **MWT** = meter walking test; **NG** = nasogastric tube; **NH** = natural history; **NIV** = noninvasive ventilation.

Introduction

Congenital myopathies (CM) are clinically and genetically heterogeneous conditions with substantial morbidity and in some instances early mortality.[1-3] Phenotypes range from severe early-onset presentations with neonatal hypotonia and (cardio) respiratory involvement to slowly progressive weakness with preserved respiratory function. Pathogenic variants in >30 genes can cause CM.[3,4] Dominant and recessive pathogenic variants in the *RYR1* gene, encoding for the principal skeletal muscle calcium release channel (RyR1) with a crucial role in excitation-contracting coupling, cause one of the most common forms of CM, known as *RYR1*-related myopathies (*RYR1*-RM), with a reported prevalence of >1:90.000.[1] The phenotypic spectrum of *RYR1*-RM is wide, with the larger cohort studies published to date supporting tentative genotype-phenotype correlations.[1,4,5] Dominant (AD) pathogenic variants in *RYR1* are commonly described in patients with the typical central core disease (CCD) phenotype, named after the characteristic histopathologic abnormality, whereas recessive (AR) pathogenic variants cause a wider clinical spectrum, with higher frequency of neonatal hypotonia, greater motor difficulties, ptosis, and respiratory and extraocular muscles involvement.[5] Histopathologic changes associated with AR *RYR1*-RM are also more variable, often comprising (multiple) cores, increased (centralized) nuclei and/or fiber-type disproportion. AD *RYR1* pathogenic variants also cause the malignant hyperthermia susceptibility (MHS) trait, a pharmacogenetic predisposition to adverse reactions to certain general anesthetics and muscle relaxants, with considerable overlap with AD *RYR1*-RM. Nonskeletal muscle symptoms, including increased bleeding tendency,[6] have been recently described in patients with *RYR1*-RM, particularly those with MHS-associated variants. Apart from few cross sectional and relatively small longitudinal studies, the long-term disease course of *RYR1*-RM is not yet clearly defined.[7-10]

Treatment for *RYR1*-RM remains largely symptomatic, although experimental therapies are starting to emerge.[11] Lack of quantifiable data on motor function and longitudinal trajectories over time, of systematic evaluation of outcome measures, and of robust data on genotype-specific natural history (NH) are major obstacles for trial readiness for this common CM.

In this study, we provide detailed retrospective cross-sectional and longitudinal NH data on a pediatric cohort of patients with *RYR1*-RM, addressing an important unmet need in the translational path concerning this relatively common group of myopathies.

Methods

This is a retrospective, cross-sectional, and longitudinal study conducted on pediatric patients affected by *RYR1*-RM. All recruited patients were followed up either at the Dubowitz Neuromuscular Centre (DNC) (located at Hammersmith Hospital until 2008 and then at Great Ormond Street Hospital) or the Neuromuscular Service at the Evelina Children's Hospital, in London, from 1992 to 2019. All patients received diagnoses of *RYR1*-RM at the UK highly specialized service (HSS) for CM and muscular dystrophies based at the DNC. Diagnosis was confirmed by the presence of pathogenic *RYR1* variants. Identification and interpretation of genetic variants and segregation analysis to determine inheritance pattern were completed at the HSS. Patients were classified as either AD or AR *RYR1*-RM based on the inheritance pattern.

Data were systematically collected from individual medical records. A predefined database template was used to ensure data collection uniformity. The following variables were longitudinally collected at each follow-up: weight, height, muscle weakness (MRC grading), Hammersmith functional motor scale,[12] Functional motor scale by Scott et al,[13] 10-meter walking test (MWT), time to rise from sitting and from lying, joint contractures, spinal deformity, Cobb angle, forced vital capacity (FVC), FVC percentage predicted (FVC%), respiratory support requirements, feeding and swallowing abilities, nasogastric tube (NG) feeding, gastrostomy, facial weakness, ophthalmoplegia, cardiac function, history of rhabdomyolysis or malignant hyperthermia, bleeding abnormalities, learning difficulties, and/or psychiatric disorders. Respiratory compromise at birth was defined as requirement for supplemental oxygen and noninvasive (NIV) or invasive ventilation during the first week/s of life. Respiratory compromise later in life was defined as FVC% <60% and/or use of NIV.

Disease severity was assessed by applying the disease severity rating scale described by Amburgey et al.[8] (Amburgey disease severity scale, or ADS-S). Because this scoring relies on knowledge of ambulatory status, the ADS-S was only applied for children >2 years (N = 67). We also created and applied a novel infant disease severity rating scale (IDS-S) based on the presence/absence of 4 features in infancy: (1) hypotonia and/or muscle weakness, (2) joint contractures, scoliosis, or developmental dysplasia of the hip (DDH), (3) respiratory compromise, and (4) feeding and/or swallowing difficulties or feeding support requirement. Each feature scored one point. An IDS-S of 1–2 was considered mild, whereas scores of

Table 1 List of *RYR1* Gene Variants Identified in the 69 Patients With *RYR1*-RM

Pt N	Variant 1	Variant 2	Inheritance	Disease severity
1	c.14692T>C p.(Ile4898Thr)		AD	
2	c.12960_12968del9 p.(Arg4321_Leu4323del)		AD	
3	c.14582G>A p.(Arg4861His)		AD	
4	c.6304G>T p.(Val2102Leu)		AD	IDS
5	***c.13013_13032del20 p.(Ala4338fs)***		AD	
6	c.11941C>T p.(His3981Tyr)		AD	
7	c.14678G>A p.(Arg4893Gln)		AD	
8	c.14678G>A p.(Arg4893Gln)		AD	
9	c.14749T>C p.(Phe4917Leu)		AD	
10	c.7354C>T p.(Arg2452Trp)		AD	
11	c.14582G>A p.(Arg4861His)		AD	
12	c.13912G>A p.(Gly4638Ser)		AD	
13	c.13912G>A p.(Gly4638Ser)		AD	
14	c.14474G>C p.(Arg4825Pro)		AD	
15	c.14588_14605del; p.(Phe4863_Asp4869delinsTyr)		AD	ADS
16	c.14779G>T p.(Val4927Ph)		AD	IDS
17	c.14779G>T p.(Val4927Ph)		AD	IDS
18	c.14680G>C p.(Ala4894Pro)		AD	
19	c.14680G>C p.(Ala4894Pro)		AD	
20	c.14680G>C p.(Ala4894Pro)		AD	
21	c.13913G>A p.(Gly4639Asp)		AD	
22	c.14582G>A p.(Arg4861His)		AD	
23	c.14582G>A p.(Arg4861His)		AD	
24	c.2537C>T p.(Ser846Leu)		AD	
25	c.14474G>C p.(Arg4825Pro)		AD	
26	c.14474G>C p.(Arg4825Pro)		AD	
27	c.9148>A p.(Val3050Ile)		AD	
28	c.13913G>A p.(Gly4639Asp)		AD	
29	c.115999C>T p.(Arg3867Cys)		AD	
30	c.7585G>A p.(Asp 2529Asn)	c.4777C>T p.(Arg 1539Trp)	AR	IDS, ADS
31	c.4405C>T p.(Arg 1469Trp)	***c.14173-2A>G***	AR	IDS
32	***c.425-1G>A***	c.14524G>A p.(Val4842Met)	AR	IDS
33	***c.3449delG p.(Cys1120fs)***	c.9457G>A p.(Gly3153Arg) c.6469G>A p.(Glu2157Lys)	AR	IDS, ADS
34	c.14524G>A p.(Val4842Met)	c.2113G>C p.(Gly705Arg	AR	
35	***c.2060_2061delTC p.(Leu687fs)***	c.4405C>T p.(Arg 1469Trp)	AR	IDS
36	***c.2060_2061delTC p.(Leu687fs)***	c.4405C>T p.(Arg 1469Trp)	AR	IDS
37	c.3877C>A p.(Pro1293Thr)	c.14939C>T p.(Thr4980Met)	AR	IDS, ADS
38	c.5030A>G p.(Asn1677Ser)	c.11752A>C p.(Thr3918Pro)	AR	IDS, ADS

Continued

Table 1 List of *RYR1* Gene Variants Identified in the 69 Patients With *RYR1*-RM *(continued)*

Pt N	Variant 1	Variant 2	Inheritance	Disease severity
39	c.10343C>T p.(Ser3448Phe)	***c.14365-2A>T***	AR	ADS
40	c.10343C>T p.(Ser3448Phe)	***c.14365-2A>T***	AR	
41	***c.2348C>A p.(Ser783Ter)***	c.4762C>T p.(Pro1588Leu)	AR	
42	c.113115G>A p.(Arg3772Gln)	c.113115G>A p.(Arg3772Gln)	AR	
43	c.113115G>A p.(Arg3772Gln)	c.113115G>A p.(Arg3772Gln)	AR	IDS
44	***c.6721C>T p.(Arg2241Ter)***	c.99999T>C p.(Leu2963Pro)	AR	IDS, ADS
45	***c.6721C>T p.(Arg2241Ter)***	c.99999T>C p.(Leu2963Pro)	AR	ADS
46	c.11798A>G p.(Tyr3933Cys)	c.12687G>T p.(Lys4229Asn)	AR	
47	***c.6721C>T p.(Arg2241Ter)***	c.325C>T p.(Arg109Trp)	AR	IDS
48	***c.6721C>T p.(Arg2241Ter)***	c.325C>T p.(Arg109Trp)	AR	
49	c.2635G>A p.(GLu879Lys)	***c.3381+1***	AR	
50	c.2635G>A p.(GLu879Lys)	***c.3381+1***	AR	
51	c.1205T>C p.(Met402Thr)	***c.11763C>A p.(Tyr3921Ter)***	AR	
52	c.13892A>G p.(Tyr4631Cys)	c.11798A>G p.(Tyr3933Cys)	AR	
53	c.13892A>G p.(Tyr4631Cys)	c.11798A>G p.(Tyr3933Cys)	AR	
54	c.13892A>G p.(Tyr4631Cys)	c.11798A>G p.(Tyr3933Cys)	AR	
55	***c.12063_12064dupCA p.(Met4022fs)***	c.11912C>T p.(Pro397Leu)	AR	
56	***c.632-2A>G***	c.9331C>T p.(Arg3111Trp)	AR	
57	***c.10347+1G>A***	***c.9686-9del p.(Ile3229Metfs*86)***	AR	IDS
58	***c.12063_12064dupCA p.(Met4022fs)***	c.14598G>C p.(Lys4866Asn)	AR	
59	***c.12063_12064dupCA p.(Met4022fs)***	c.14598G>C p.(Lys4866Asn)	AR	IDS
60	c.11798A>G p.(Tyr3933Cys); c.4711A>G p.(Ile1571Val); c.10097G>A p.(Arg3366His)	***c.99943delC p.(Ser2948fs)***	AR	
61	c.13903G>C p.(Glu4635Glc)		de novo AD	ADS
62	c.14582G>A p.(Arg4861His)		de novo AD	
63	c.12083C>T p.(Ser4028Leu)		de novo AD	
64	c.14422T>A p.(Phe4808Ile)		de novo AD	
65	c.14581C>T p.(Arg4861Cys)		de novo AD	IDS
66	c.14581C>T p.(Arg4861Cys)		de novo AD	
67	c.14423T>A p.(Phe4808Tyr)		uncertain	
68	c.14126C>T p.(Thr1709Met)		uncertain	IDS, ADS
69	c.14741G>C p.(Arg4914Thr)		uncertain	

Abbreviations: AD = autosomal dominant; ADS = Amburgey severity scale >5; AR = autosomal recessive; IDS = infant severity scale >3.
Hypomorphic variants are indicated in bold italics.

3–4 were considered severe. Severity scores were applied using previously collected patient data.

Descriptive statistics are presented for all measures; mean and standard deviation were used for normally distributed data, and median and interquartile range for skewed data. Categorical data were summarized as frequency and percentage. We assessed the linear correlation between continuous variables using the Pearson correlation, and Mann-Whitney *U* test was used to compare medians between groups for skewed variables. The Fisher Exact Test was used to compare frequencies of categorical variables between

Table 2 Clinical Features of 72 Patients With *RYR1* Gene–Related Myopathy

	Total number patients (n = 69)	AD patients (n = 29)	AR patients (n = 31)	*p* Value
Females/Males (% females)	38/31 (55%)	13/16 (45%)	18/13 (58%)	0.31
Median age at first assessment, y	4.0	4.9	2.0	**0.02**
Median age at the last assessment, y	10.8	12.2	9.6	**0.66**
Mean follow-up period, y	6.2 (4.2)	6.1 (4.0)	6.0 (4.5)	0.94
Mean age at presentation, y	0.7 (1.2)	1.1 (1.0)	0.6 (1.4)	**0.13**
Prenatal features	27/65 (42%)	8/28 (29%)	14/28 (50%)	0.10
Reduced foetal movements	21/60 (35%)	4/24 (17%)	13/27 (48%)	**0.02**
Oligohydramnios	3/62 (5%)	3/26 (12%)	0/27 (0%)	—
Polyhydramnios	9/62 (15%)	3/26 (12%)	5/27 (19%)	0.17
Preterm birth	11/61 (18%)	4/26 (15%)	5/28 (18%)	0.81
Neonatal Features	37/67 (55%)	8/28 (29%)	22/31 (71%)	**<0.01**
Hypotonia at birth	27/67 (40%)	5/28 (18%)	18/31 (58%)	**<0.01**
Contractures at birth	12/67 (18%)	3/28 (11%)	6/31 (19%)	0.48
Feeding difficulties at birth	27/67 (40%)	4/28 (14%)	20/31 (65%)	**<0.001**
Respiratory difficulties at birth	15/67 (22%)	3/28 (11%)	11/31 (36%)	**0.03**
Motor abilities at first assessment (patients aged >2 y)				
Running	20/50 (40%)	14/25 (56%)	7/18 (39%)	0.41
Independent ambulation	19/50 (38%)	10/25 (40%)	7/18 (39%)	
Ambulant with support	6/50 (12%)	3/25 (12%)	2/18 (11%)	
Nonambulant	5/50 (10%)	0/25 (0%)	2/18 (11%)	
Best motor abilities (patients aged >2 y)				
Running	37/65 (57%)	18/28 (64%)	18/28 (64%)	0.31
Independent ambulation	18/65 (28%)	9/28 (32%)	5/28 (18%)	
Ambulant with support	7/65 (11%)	1/28 (4%)	3/28 (11%)	
Nonambulant	3/65 (5%)	0/28 (0%)	2/28 (7%)	
Motor abilities at the last follow-up (patients aged >2 y)				
Running	34/65 (52%)	18/28 (64%)	15/28 (54%)	0.38
Independent ambulation	17/65 (26%)	8/28 (28%)	7/28 (25%)	
Ambulant with support	8/65 (12%)	2/28 (7%)	3/28 (11%)	
Nonambulant	6/65 (9%)	0/28 (0%)	3/28 (11%)	
Axial weakness	58/69 (84%)	24/29 (83%)	26/31 (84%)	0.91
Facial weakness	49/69 (71%)	14/29 (48%)	26/31 (84%)	**<0.01**
Ptosis	20/69 (29%)	4/29 (14%)	14/31 (45%)	**<0.01**
Extraocular muscles involvement	13/69 (19%)	0/29 (0%)	12/31 (39%)	**<0.001**
Scoliosis	21/69 (30%)	9/29 (31%)	7/31 (23%)	0.46
Scoliosis surgery	8/69 (12%)	3/29 (10%)	4/31 (13%)	0.76
Spinal rigidity	12/69 (17%)	0/29 (0%)	9/31 (29%)	**<0.01**
Noninvasive ventilation	10/69 (14%)	0/29 (0%)	8/31 (26%)	**<0.01**

Continued

Table 2 Clinical Features of 72 Patients With *RYR1* Gene–Related Myopathy *(continued)*

	Total number patients (n = 69)	AD patients (n = 29)	AR patients (n = 31)	*p* Value
Respiratory compromise[a]	15/69 (22%)	1/29 (3%)	10/31 (32%)	**<0.01**
Gastrostomy/Nasogastric tube feeding	11/69 (16%)	0/29 (0%)	10/31 (32%)	**<0.01**
Weight Z-score at first evaluation (average age in years (SD); n of patients)	−0.9 (1.7); n = 35	−0.1 (1.3); n = 16	−1.6 (1.7); n = 19	**<0.01**

Abbreviations: AD = autosomal dominant; AR = autosomal recessive; N = number.
Statistically significant *p* values < 0.01 are indicated in bold.
[a] FVC% <60% or by use of noninvasive ventilation.

groups. We fitted mixed effects regression models to assess the longitudinal trend for motor time tests and FVC% with age, and the annual change was estimated for each outcome. A *p* value < 0.01 was considered significant. We used Stata and SPSS software for statistical analyses.

Standard Protocol Approvals, Registrations, and Patient Consents

This work does not require approval from an ethics committee, and it has been reviewed and approved at Great Ormond Street Hospital as a Clinical Audit in line with the principles of conduct outlined in Great Ormond Street Hospital Trust Clinical Audit Policy (Reg N 3286).

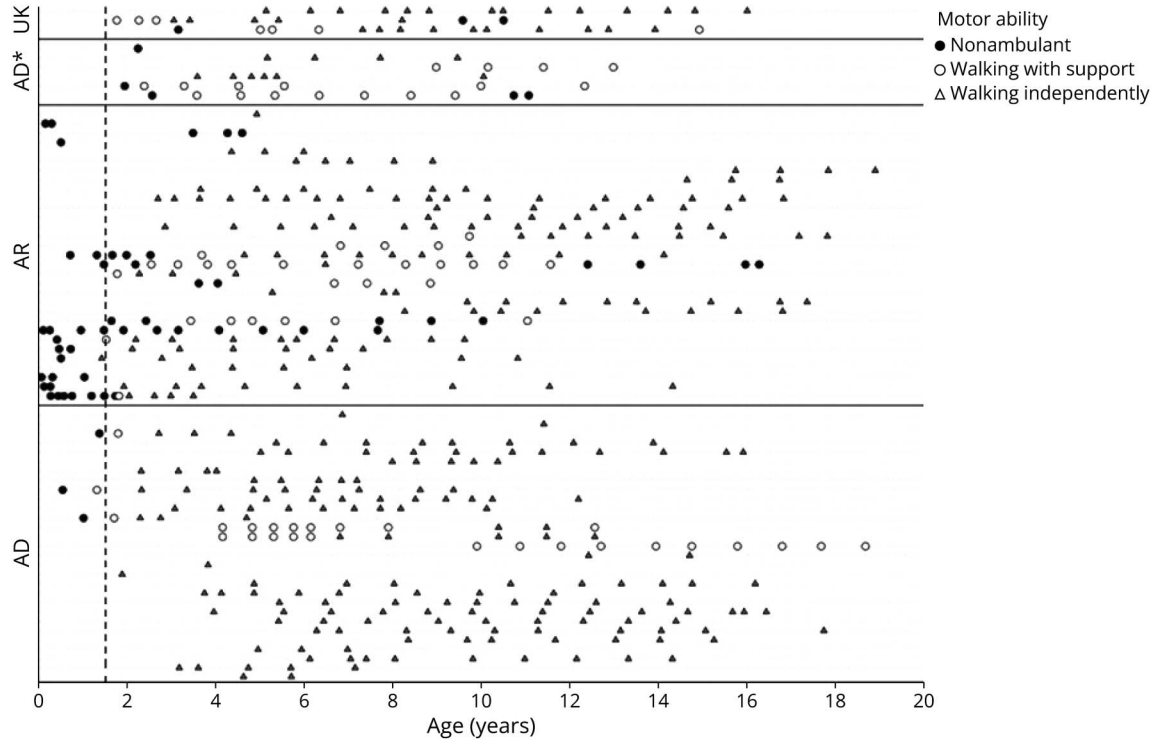
Data Availability

Deidentified participant clinical data not included in the article are available on reasonable request by qualified investigators to the corresponding author.

Results

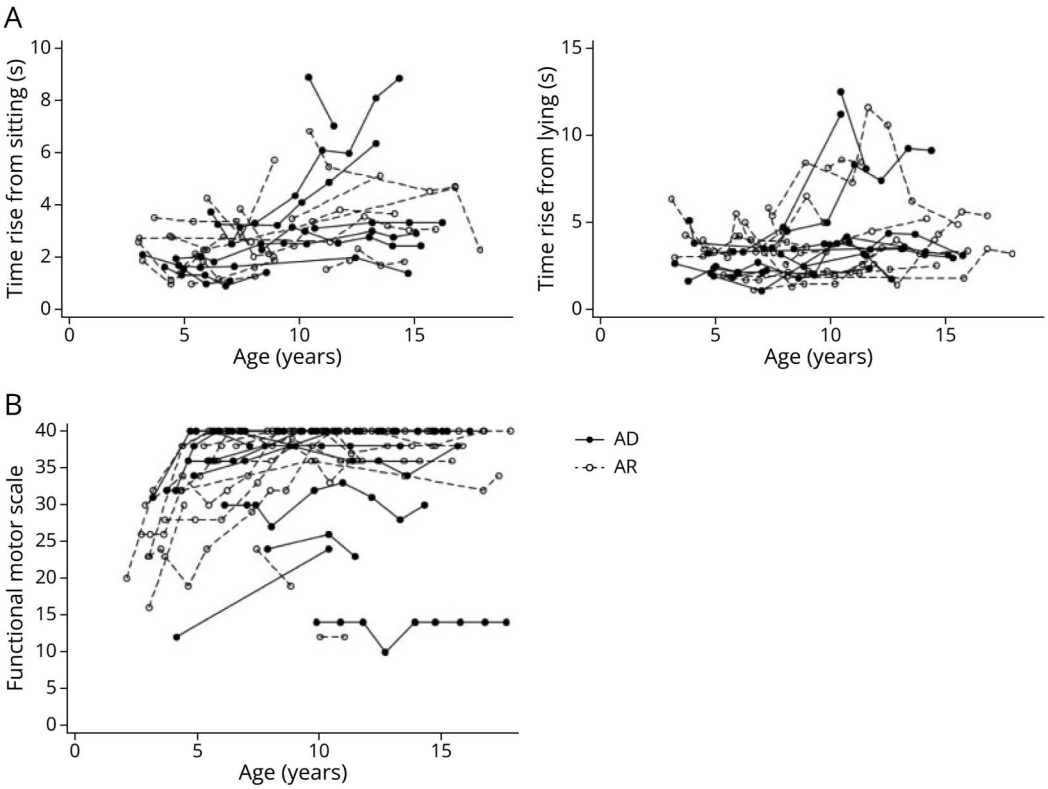
The study cohort included 69 patients (38 women, 55%) from 53 unrelated families. Cross-sectional and longitudinal data was available for 63 patients, with cross-sectional data only available for additional 6 patients. Details of the pathogenic *RYR1* gene variants are indicated in Table 1.

Figure 1 Longitudinal Observational Data on Gross Motor Abilities in Patients with *RYR1*-RM



Each horizontal line indicates single patients. Symbol on lines indicates different assessments in each patient. Patients are distributed according to inheritance, starting from the bottom: patients with autosomal dominant inheritance (AD), autosomal recessive (AR), and apparent de novo dominant (AD*) and uncertain inheritance (Uk). Full circles represent nonambulation; white circle represent ability to walk with support; grey triangle ability to walk independently. The dashed vertical line at 18 months indicates WHO thresholds for attaining independent sitting and walking alone in 99% of children.

Figure 2 Longitudinal Analysis of Motor Ability Tests



(A) Spaghetti plots representing timed rise from sitting and from lying on the floor in AD and AR patients. (B) Spaghetti plots representing individual functional motor scale scores for the entire cohort of patients with *RYR1*-RM at all available time points.

Twenty-nine patients (42%) had AD *RYR1*-RM, and 31 (45%) AR *RYR1*-RM. Segregation analysis in 6 patients suggested de novo origin of a dominant variant (de novo AD patients). Inheritance pattern was unclear in 3 patients because of limited availability of family members for segregation analysis. These 9 patients were not included in the statistical analysis.

The number of visits ranged from 1 to 18 for each patient (median 6.0). The mean clinical follow-up period was 6.2 years (range 0–14.8 years). The median age at the first and last assessment was 4.0 years and 10.8 years, respectively (range 4 weeks–15.7 years and 4 months–22.9 years). Table 2 summarizes demographics and clinical features of the full cohort.

Onset

Age of symptoms onset was available for 67 patients. Symptoms were present at birth in 37 patients (55%), before 12 months in 5 (7%), between 1-2 years in 21 (31%), and between 3-6 years in further 4 patients (6%). Mean age at presentation was 0.7 years (range 0–7 years) for the whole cohort, 1.1 years in AD patients, 0.6 years in AR, and 0.2 years in AD de novo and 0 in those with uncertain inheritance ($p = 0.13$).

Prenatal and Neonatal Symptoms

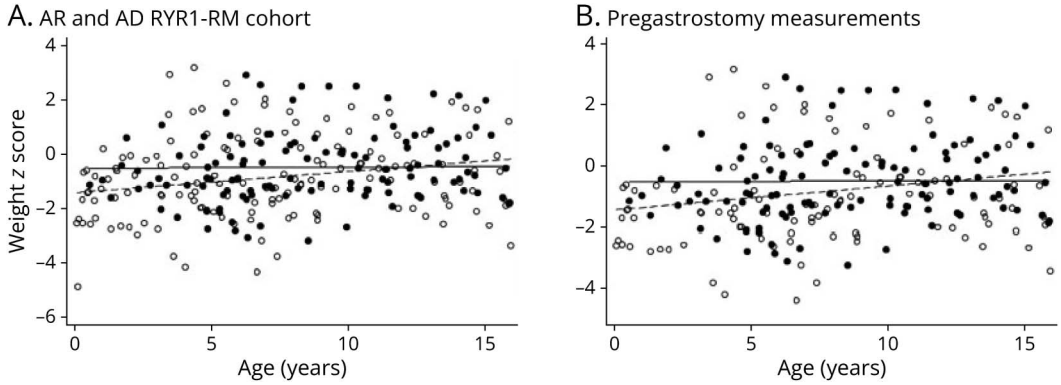
Data on prenatal/neonatal features was available for 65 patients. Prenatal features were present in 27 patients, with no significant difference among genetic groups (Table 2). Preterm birth (11 patients) and breech presentation (11 patients) were frequent.

Symptoms at birth were reported in 37 patients and more common in AR patients ($p < 0.01$) (Table 2). Hypotonia, feeding difficulties, and respiratory compromise at birth were particularly common. Contractures (including neck, shoulder, elbow, wrist, thumb, hip, knee, and ankles) were present in 12 patients across all subgroups. DDH was recorded in 13 patients. Extraocular muscle involvement was noted at birth in 2 AR patients.

No patients had a history of hydrops fetalis, pterygium, or cleft palate. Dysmorphic features included high arched palate (15 patients), macro/microcephaly, micrognathia, and malocclusion in isolated patients only.

Hypotonia was present in 3 of 6 patients with apparent de novo AD variants. None showed respiratory problems, and 2 had feeding difficulties. Three de novo AD patients had DDH, and 2 had multiple contractures at birth. Facial weakness,

Figure 3 Weight Z-Scores



Trend lines representing the 2 subgroups of dominant (black circles) and recessive (white circles) patients with *RYR1*-RM (A). In (B), only pregastrostomy values were included. Z scores are derived from a normative UK population. A Z-Score of 0 corresponds to the 50th centile, a Z-score of −1 corresponds to the 26th centile, a Z-Score of −2 corresponds to the 2.5th centile, and a Z-score of −3 corresponds to the 0.3rd centile.

scoliosis, and hiatus hernia at birth were noted in 1 de novo AD patient, respectively.

Gross Motor Function

At the first assessment of the 50 patients >2 years, 45 were ambulant with/without support (age range 2.3–15.7 years; median 5.2 years). Age at the achievement of ambulation was not available. Additional 15 patients attained the ability to walk with/without support during the follow-up. Three walked by age 18 months (Figure 1).

We analyzed functional abilities of patients >2 years at baseline, at the last appointment, and at the time of their best motor ability (Table 2). Ten patients >2 years (10/65, 15%; 1/28 AD, 5/28 AR, 4/6 de novo AD, and 0/3 unclear inheritance) never walked without support. Three patients lost the ability to run, and 4 lost independent ambulation (4/55; 7%). In particular, 1 AR patient and 1 with uncertain inheritance lost ambulation at age 8 and 11 years, respectively. The remaining 1 AD patient and 1 with uncertain inheritance were ambulant with support at age 12 and 15 years. An additional AD patient remained ambulant with full leg orthoses or gaiters until age 8 years. Four of the 12 children with DDH older than 2 years did not achieve independent ambulation.

Overall, 43% of the 14 patients who did not walk/lost ambulation had AR inheritance, 29% de novo AD, 14% AD, and 14% uncertain inheritance (representing 21%, 67%, 7%, and 67% of AR, de novo AD, AD, and uncertain inheritance cohorts, respectively). We observed better motor abilities in the AD vs AR + de novo AD subgroups, both at the time of best mobility and at the last appointment ($p < 0.01$).

Longitudinal analysis of 10 MWT, timed rise from sitting, and timed rise from lying was available for 43, 33, and 41 patients, respectively (total of 197, 113, and 150 observations, each). An analysis of the 10 MWT in the whole cohort indicates a yearly change of −0.28 units/y, 95% CI (−0.40 to −0.17) ($p < 0.001$). A slow decline in early age was apparent in both AD and AR groups, with AR patients being more stable after the age of 7–8 years. Timed rise from floor sitting increased 0.19 s/y, 95% CI (0.04–0.34) in AD patients vs 0.05 s/y, 95% CI (−0.11 to 0.21) in AR patients ($p = 0.22$). Similarly, timed rise from lying increased 0.31 s/y, 95% CI (0.12–0.49) in the AD patients ($p < 0.001$) vs 0.05 s/y, 95% CI (−0.12 to 0.22) in the AR group ($p = 0.05$) (Figure 2A).

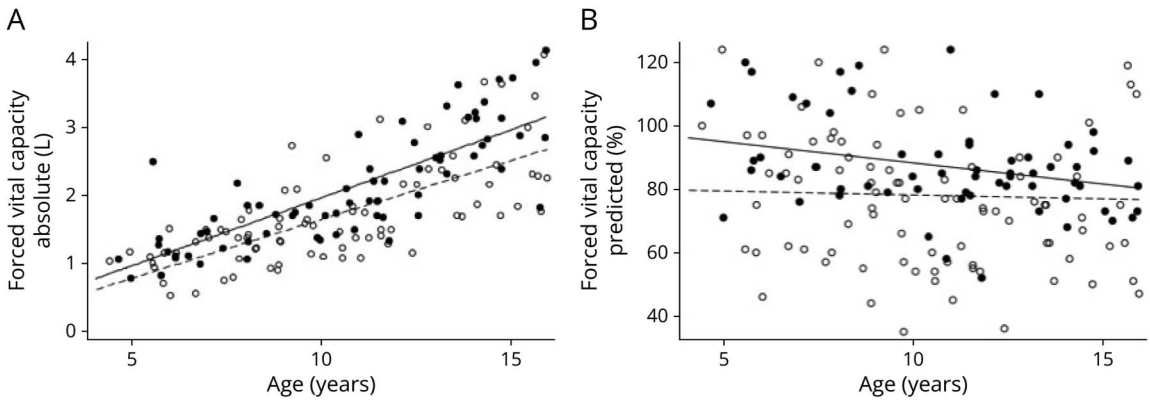
The Functional motor scale by Scott et al.[13] was administered to 43 patients, of which 39 had ≥2 sequential evaluations at >12 months apart (total 212 assessments). Analysis showed a large ceiling effect with no real trend (Figure 2B). The Hammersmith functional motor scale[12] was available for 15 patients for a total of 65 longitudinal assessments. Owing to the limited number of evaluations, statistical analysis was not completed (data not shown).

Distribution of Weakness

At the last assessment, all patients but one showed proximal more than distal weakness affecting the lower more than the upper limbs. Upper limb weakness was reported in 68 patients. Fifty-eight patients had axial weakness (24/29 AD, 26/31 AR, 5/6 de novo AD).

Facial weakness was observed in 49 patients (14/29 AD vs 26/31 AR, $p < 0.01$) with 4 of 29 AD vs 14 of 31 AR patients having ptosis ($p =< 0.01$) and 0 of 29 AD vs 12 of 31 AR patients having extraocular muscle involvement ($p = 0.001$). All 6 de novo AD patients had facial weakness, but only 1 had ptosis and none had extraocular muscle involvement. One additional patient with uncertain inheritance had facial weakness, ptosis, and complete ophthalmoplegia.

Figure 4 Respiratory Function



(A) Scatter plots representing absolute values of forced vital capacity for the 2 subgroups of dominant (black circles, continuous trend line) and recessive patients with *RYR1*-RM (white circles, dotted trend line). (B) Scatter plot representing forced vital capacity % predicted for the 2 subgroups of dominant (black circles, continuous trend line) and recessive patients with *RYR1*-RM (white circles, dotted trend line).

Feeding

Swallowing and/or feeding difficulties were recorded in 23 patients at any time point and were more common in AR patients (3/29 AD vs 17/31 AR, $p < 0.01$; 1/6 de novo AD). Twenty-two patients had failure to thrive at any time point. A total of 327 weight measurements were available for 65 patients. Mean weight Z-scores at the first available assessment were higher in AR (−0.14, 95% CI (−0.84 to 0.57)) vs AD patients (−1.56, 95% CI (−2.36 to −0.76)) ($p < 0.01$) (Figure 3A). Weight trajectories showed an increase of 0.04 units/year in Z-score (95% CI (−0.005 to 0.08) $p = 0.09$). Considering only measurements before gastrostomy insertion, weight Z-score increased by 0.02 units/years (95% CI (−0.02 to 0.06) $p = 0.42$) (Figure 3B). Weight Z-scores increased in particular in the AR patients (0.05, 95% CI (−0.008 to 0.11) $p = 0.09$), whereas in the AD patients, these were more stable over time (0.003, 95% CI (−0.06 to 0.06) $p = 0.93$).

Nine patients required NG feeding at any time point, and 2 still required NG feeds at the last appointment at age 0.3 and 0.5 years. Eleven patients had a gastrostomy inserted (10/31 AR vs 1/6 de novo AD; $p = 0.001$) at a median age of 0.5 years (range 0.1, 9.7 years; median age in AR group 1.1 years). Eight patients were gastrostomy-fed at the last assessment, and 3 had their gastrostomy reverted at 3.19, 10.82, and 12.8 years, respectively (median 10.8 years).

Orthopedic Complications

Spine X-ray results were available for 15 patients (total 66 films). One AR and 1 de novo AD patient had scoliosis at birth. During the follow-up, 21 (29%) patients developed scoliosis and 12 (17%) had spinal rigidity documented. No significative difference was noted between AR and AD patients for scoliosis. Spinal rigidity was more frequent in AR patients (9/31 AR vs 0/29 AD $p = 0.01$; 2/6 de novo AD). Eight patients had spinal surgery (4 with magnetic controlled growth rods) at a median age of 8.2 years (range 4.2–15.5 years). Median Cobb angle at the time of surgery was 58°. No severe adverse reactions were documented for these procedures.

Thirty-five patients had contractures, 24 in the lower limbs and 11 in both upper and lower limbs. Hyperlaxity at any time point was documented in 43 patients.

Respiratory Function

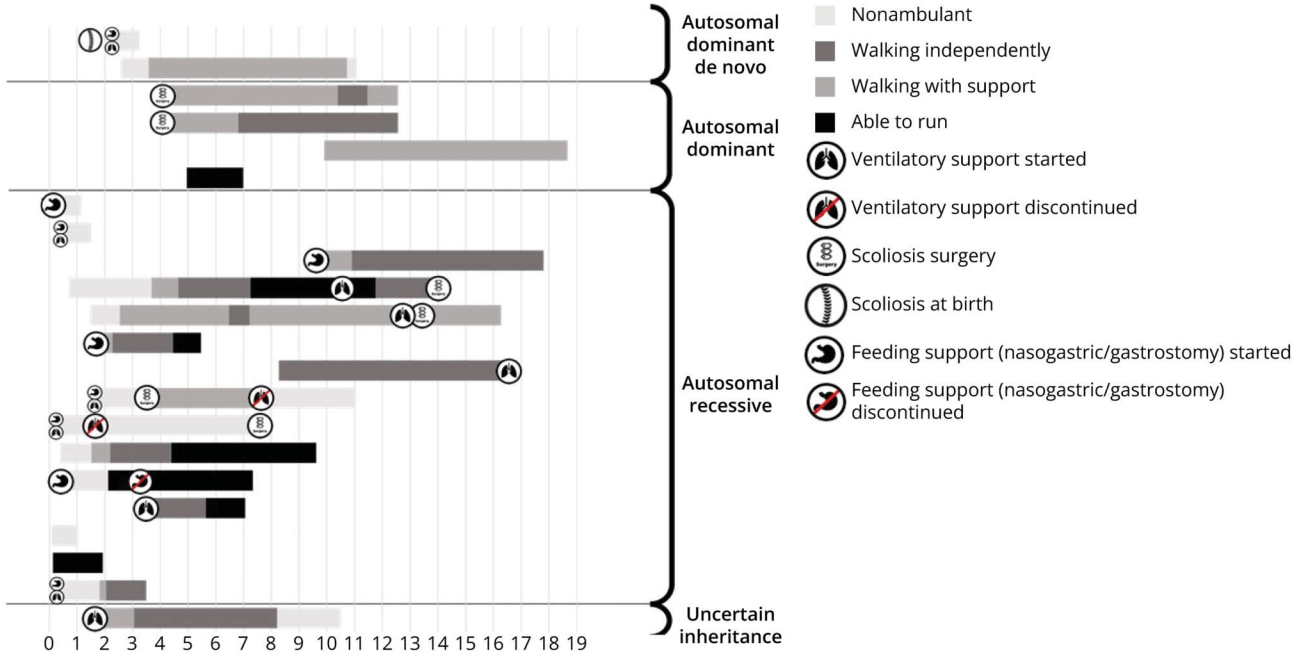
A total of 177 FVC recordings were available for 45 patients (age range 4.6–18.9 years). At the first assessment, 2 patients had FVC <40% at 8.2 and 9.7 years. FVC% <60% or <40% at any time point was observed in 11 and 5 patients, respectively, at an average age of 9.7 and 11.1 years. An analysis of absolute FVC values for the entire cohort showed an annual increase of 0.17 L/y (95% CI (0.15–0.20) $p < 0.001$) but a decrease in FVC% of −0.89% year (95% CI (−1.64 to −0.14) $p = 0.02$) (Figure 4A). Decrease in FVC% was greater in the AD subgroup (−1.3, 95% CI (−2.5 to −0.2) $p = 0.02$), whereas it remained more stable in the AR patients (−0.2, 95% CI (−1.3 to 0.9) $p = 0.66$) (Figure 4A). However, the difference in slopes between the 2 groups was not significant ($p = 0.18$). There was no difference in FVC change/year before or after spinal surgery (FVC absolute change/year +0.18 L/y, $p < 0.001$, FVC% change/year −0.93% year, $p = 0.02$).

Ten patients (8 AR, 1 de novo AD, 1 uncertain inheritance) needed NIV at any time point, at a median age of 2.5 years (range 0–16.8 years) (Table 2). Four of these 10 patients (3 AR, 1 de novo AD) needed day and night-time NIV at some point. NIV use was reduced or stopped at 6 months of age in 2 AR patients. No patient had a tracheostomy.

Other Clinical Features

Four patients had cardiac complications, including septal defects (2 patients), ventricular bigeminy rhythm (1 patient), and mild left ventricular hypertrophy (2 patients). Profuse sweating

Figure 5 Clinical Severity



Disease severity progression in selected patients with infantile severity score >3 or Amburgey severity score >5. Each horizontal bar indicates single patients. Patients are grouped according to inheritance pattern. Gross motor function and comorbidities are indicated for each patient as in legend.

and heat intolerance were reported in 4 patients (3 AD, 1 uncertain inheritance). Six patients (3 AD, 2 AR and 1 with uncertain inheritance) had psychiatric/neurodevelopmental features including behavioral difficulties, social communication disorder, or low mood/anxiety. Learning difficulties, of different severity, were reported in 9 patients.

Rhabdomyolysis and/or MH reaction were not observed in this cohort of patients. There was also no history of other *RYR1*-related crisis events. Creatine kinase levels were available for 44 subjects and were normal throughout. One patient had a history of excessive bleeding, and 1 had menstrual abnormalities.

Survival

All patients were alive at the time of this study. However, survival data after transition to adult services were not available.

Severity Analysis

To better investigate determinants for disease progression and to identify possible genotype-phenotype correlations, we applied 2 different severity scales: (1) a new scale, IDS-S, applied in infancy, with severity criteria arbitrarily established based on the presence of neonatal hypotonia, weakness, contractures, respiratory failure, and/or feeding difficulties and (2) the ADS-S, applied to patients >2 years of age.[8]

In total, 22 of 69 (32%) patients had severe scores on either of these scales. In particular, 17 of 69 (25%) patients scored ≥3 points in the IDS-S (3 AD, 12 AR, 1 de novo AD, 1 with uncertain inheritance), whereas 10 patients (14%; 1 AD, 7 AR, 1 de novo AD, 1 with uncertain inheritance) had an ADS-S score ≥5. Five AR patients and 1 with uncertain inheritance scored severe in both scales (Figure 5).

Among the 22 severe patients, 5 (23%) were nonambulant. Three of these scored severe on the IDS-S, and 4 on the ADS-S (Figure 5). Three of the 4 patients who lost independent ambulation during the follow-up had severe scores on either IDS-S (2 patients) or ADS-S (2 patients).

Muscle MRI

Muscle MRI was performed in 19 patients and showed predominant anterior thigh (vastus lateralis, sartorius, adductor longus) and glutei involvement (data not shown). Four patients with a mild phenotype had normal muscle MRI (2 AD, 1 AR and 1 de novo AD; age at MRI being 5 years in 2 patients and 6 in further 2).

Histopathology

Muscle biopsy was available for 41 patients. Fourteen patients had features in keeping with a diagnosis of CCD (5 AD, 5 AR, 2 de novo AD, 2 uncertain inheritance), 4 of whom (3 AR, 1 uncertain inheritance) also showed an increase of central nucleation. Minicore/multiminicore myopathy was reported in 10 patients (3 AD, 5 AR, 2 de novo AD), and increased central nucleation in 13 patients (1 AD, 9 AR, 1 de novo AD, 2 uncertain inheritance). Fiber-size disproportion and/or type 1 predominance was observed in 19 patients (3 AD, 12 AR, 3 de novo AD, 1 uncertain inheritance).

Genetics

A total of 64 pathogenic *RYR1* variants were found in this cohort (Table 1). Variants predicted to abolish or decrease production of the protein (nonsense, frameshift, and splice site variants) were considered hypomorphic, whereas variants predicted to result in full length protein with abnormal function (missense and small in-frame insertion/deletion) were classified as nonhypomorphic. Fifty variants (78%) were nonhypomorphic (48 missense, 2 in frame ins/del variants) and 15 variants (23%) were hypomorphic. Ten AR patients were homozygous or compound heterozygous for 2 nonhypomorphic variants, 1 patient carried 2 hypomorphic variants, whereas the remaining 20 (65%) AR patients had a combination of a hypomorphic and a nonhypomorphic variant. Twelve of the 18 AR patients who had either a severe score on the severity scales or who were not ambulant had at least one hypomorphic allele (66%). The single AR patient with 2 hypomorphic alleles scored severe on the IDS-S and was too young to be scored on the ADS-S. We assessed the location of nonhypomorphic variants relative to the 3 principal mutation hot spots of the gene, MH1 (amino acid residues 35–614), MH2 (amino acid residues 2,163–2,458), and MH3 (amino acid residues 4,550–4,940): 20 variants were found in MH3, 2 in MH1, and 3 in MH2 domain. Among these, 12 variants (41%) were associated with a severe phenotype or lack of ambulation vs a rate of 22% among all variants found.

Discussion

RYR1-RM are the most common form of CM worldwide,[1] but there is lack of clear knowledge regarding their long-term NH. Clarification of disease progression, associated complications, and timing of interventions will help to refine the standards of care and management and identify disease-specific milestones for evaluating emerging novel therapies. A number of therapeutic approaches, including modification of *RYR1* Ca2+ release, use of chemical chaperones, restoration of mRNA reading frame, stop codon suppression, exon skipping, and/or selective silencing, are in the preclinical phase and/or close to clinical application.[14-16] After observation of increased oxidative stress in *RYR1*-RM, a 6-month NH study and a first clinical trial with antioxidant N-acetylcysteine was completed on 33 patients.[17,18] This study showed no decrease in oxidative stress in treated patients, and evidenced stable disease course in ambulatory patients,[18] highlighting the need for longer prospective, longitudinal studies.

We present the largest cross-sectional and retrospective longitudinal NH analysis of a pediatric cohort of patients with *RYR1*-RM. This study investigated ambulant and nonambulant cohorts, with similar numbers of AR and AD patients. Our study presents the longest follow-up period of patients with *RYR1*-RM reported so far (median 6.2 years, up to 14.8 years). Our results provide detailed information on relevant features at onset, and the long-term course with a particular emphasis on motor function, respiratory and bulbar involvement, and orthopedic complications. We also confirm the wide phenotypic spectrum and clinical variability both between and within the AD and AR subgroups.[5,8,9]

We highlight a higher prevalence of prenatal and neonatal features, in particular hypotonia and respiratory difficulties, in AR patients with *RYR1*-RM (Table 2). Of note, although neonatal features were also frequent in apparent de novo AD patients, extraocular muscle involvement at birth was only reported in AR patients, suggesting this as a distinguishing feature. However, because this feature is not always detected at birth, the absence of extraocular muscle involvement at birth might not a reliable indicator of inheritance pattern.

Previous reports indicated worse ambulatory function in AR patients.[2,5,8] Our current study indicates relevant motor delay in 80% of patients (Figure 1), lack/loss of independent ambulation in 21%, with higher prevalence of patients who never walked (15%), compared with those losing this ability later in life (7%). We also demonstrated worse ambulatory outcomes in de novo AD and AR patients compared with AD patients, similar to what was reported in smaller cohorts of patients with severe neonatal onset[19] (Figures 1 and 5). Nonambulant patients also showed higher prevalence of severe contractures at birth (40%), scoliosis (60%), and respiratory complications (70%), suggesting an overall more severe disease course. The high frequency of nonambulation in children with DDH (4/12 patients) highlights this as an additional factor affecting ambulatory function.

The previous NH study showed disease stability over 6 months in ambulant patients.[10,20] The functional motor scale applied in our cohort[13] is commonly used at our center in patients with CM or congenital muscular dystrophies[21] but has not been previously validated in patients with *RYR1*-RM. Although the analysis of this functional scale showed no clear trends, timed tests showed a more stable course in older, ambulant AR children compared with AD ones, perhaps suggesting a more progressive proximal weakness in AD patients (Figure 2A). Scoliosis at birth was noted in 1 AR and 1 de novo AD patient, and this may perhaps represent a feature of a more severe phenotype, as also suggested by their nonambulatory status.

One of the most significant findings of this study is the identification of respiratory compromise in >20% of patients, with 14% needing NIV at any time point. For the first time, we describe annual change of FVC values in AR and AD patients and provide evidence of greater progression in FVC% in the AD vs AR subgroup (annual change −1.4% vs −0.3%; Figure 4A). These results highlight the importance of respiratory monitoring in patients with *RYR1*-RM and suggest FVC% as possible outcome measure for therapeutic studies, in particular in AD patients. Our studies also show that the very modest rate of respiratory decline in both the AD and AR cohorts would make stabilization of decline an outcome measure requiring several years before reaching a

clinically significant value. The scenario could be different for drugs that would lead to an improvement of the respiratory muscle strength.

In addition to failure to thrive, our data emphasize a higher need for NG/PEG feeds and lower weight Z-scores in AR patients, with slow improvement over the subsequent years, reaching values similar to AD patients in the midteens (Figure 3). We also note intermittence of NG feed requirements, possibly because of spontaneous weight improvement. Of note, during the preparation of this manuscript, 2 further patients with *RYR1*-RM are awaiting the removal of PEG (data not shown). This finding, as well as the reduced NIV need beyond infancy and the improvement in severity scores in some patients, confirms a somewhat "inverse disease course" previously reported in particular in AR patients with specific ethnicities.[22,23] These findings may inform management and prognostication in infancy, as well as better design future therapeutic trials.

To better describe different severities, we applied 2 disease severity scales in infancy and later childhood. Of note, none of these scales reflected ambulatory ability, with 6 of 10 nonambulant patients scoring mild on both scales. This observation may reflect some lack of granularity of these scales, warranting further studies to clarify their prognostic validity.

A previous review of 103 *RYR1*-AR patients showed that hypomorphic variants are more common in severe presentations and non-CCD myopathy.[8] An analysis of disease severity in our cohort highlights the more pronounced clinical severity in AR patients, in particular at birth, with 58% of AR patients being severe or not achieving ambulation. However, although hypomorphic variants were indeed more common in AR patients, our analysis did not suggest a strong correlation between the type of variant and/or their severity, with 67% of AR patients with either severe or nonsevere AR phenotype harboring hypomorphic variants (Table 1). Conversely, we confirm higher occurrence of missense variants (both in AR and de novo AD patients in particular with more severe phenotypes) in the MH3 hotspot as further supporting previously described tentative genotype-phenotype correlations.[8]

This study has intrinsic limitations. Data collection was retrospective, and there was missing information at some time points. This is not unexpected for retrospective studies spanning a long period of time and may have affected the statistical power of the analysis. We cannot comment on reasons for missing data, much of it is likely to be missing at random, but for some measures, it may be related to disease severity. Hence, this may have introduced some systematic bias. In addition, disease progression in adult life was not explored.[19,24,25] We observed similar numbers of AR and AD patients, but higher prevalence of AD *RYR1*-RM were reported in adolescents and adult patients.[24] We did not include patients with isolated MHS and *RYR1*-related rhabdomyolysis.[25] However, the results of this study provide new knowledge on long-term disease progression in *RYR1*-RM that may inform management and essential milestones that could be exploited in the design of future therapeutic interventions. Prospective, long-term longitudinal studies on larger cohorts also including adult patients are needed to validate these observations. Additional motor functional scales, targeted to younger, less mobile populations (i.e., CHOP intend, MFM20), and respiratory tools need also to be investigated for their suitability to assess progression at an earlier age.

In summary, this retrospective NH study provides for the first time detailed information regarding the full clinical spectrum and long-term disease course in pediatric patients with *RYR1*-RM. The presented data provide novel insights into this common CM and relevant differences based on modes of inheritance of *RYR1* variants, helping to pave further the way toward future therapeutic interventions.

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Continued

Appendix *(continued)*

Name	Location	Contribution
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