










The etiology of rhabdomyolysis: an interaction between genetic susceptibility and external triggers

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Background and purpose: Rhabdomyolysis is a medical emergency characterized by acute skeletal muscle breakdown with a sudden rise and subsequent fall of serum creatine kinase (CK) levels. Rhabdomyolysis events are provoked by exposure to external triggers, possibly in combination with an increased genetic susceptibility. We aimed to describe comprehensively the external triggers and potentially pathogenic genetic variants possibly implicated in increased rhabdomyolysis susceptibility.

Methods: We performed a retrospective single-center study, including a total of 1302 patients with an acute CK level exceeding 2000 IU/l.

Results: Anoxia was the most frequently reported trigger (40%). A subset of 193 patients were clinically suspected of an underlying genetic disorder (recurrent episodes, a positive family history, very high or persistently increased CK levels). In 72 of these patients, an unequivocal genetic defect was identified. A total of 22 genes with pathogenic variants were identified, including 52 different variants. Of those, 11 genes have been previously associated with rhabdomyolysis (*ACADVL*, *ANO5*, *CPT2*, *DMD*, *DYSF*, *FKRP*, *HADHA*, *PGM1*, *LPINI*, *PYGM*, *RYR1*). Eleven genes are probably implicated in increased susceptibility (including *AGL*, *CAPN3*, *CNBP*, *DMPK*, *MAGT1*, *ACADM*, *SCN4A*, *SGCA*, *SGCG*, *SMPD1*, *TANGO2*).

Conclusion: These findings suggest that the spectrum of genetic susceptibility for rhabdomyolysis has not yet been completely clarified. With the increasing availability of next-generation sequencing in a diagnostic setting, we expect that in more cases a genetic defect will be identified.

Introduction

Rhabdomyolysis is a complex condition relevant to many medical disciplines, involving the rapid dissolution

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of damaged or injured skeletal muscle. This disruption of skeletal muscle integrity leads to the direct release of intracellular muscle components, including myoglobin, creatine kinase (CK), aldolase, lactate dehydrogenase and electrolytes, into the circulation and extracellular space. Clinical manifestations range from a largely asymptomatic illness with isolated serum CK level elevation (hyperCKaemia) to a life-threatening condition with profound myoglobinuria, often leading to acute renal

failure. Definitions vary, but rhabdomyolysis is most commonly defined as a clinical syndrome of severe myalgia, muscle weakness and swelling, with sudden elevation of CK levels, with or without the presence of myoglobinuria, and subsequent fall of CK levels.

Three retrospective cohort studies in a hospital setting, performed in 2005, 2009 and 2012, described the demographics and different causes of rhabdomyolysis. All three studies, performed when diagnostic genetic testing was not yet widely available, focused on external triggers as a cause for the rhabdomyolysis event and identified exogenous toxins as the most frequently reported trigger, followed by traumatic muscle injury [1–3]. However, recent reports suggest that rhabdomyolysis events can be attributed to a combination of certain environmental factors (e.g., strenuous exercise and/or febrile infection) and a predisposing genotype [4]. A combination of external triggers and genetic predisposition may thus be required for an individual to exceed the threshold for developing rhabdomyolysis (Fig. 1), and the majority of rhabdomyolysis events may therefore have to be considered multifactorial. To identify episodes of rhabdomyolysis suggestive of an underlying genetic susceptibility, we have introduced the acronym RHABDO (Box 1) [5,6]. This acronym was developed based on an extensive literature review and has been adopted in a recent review on exertional rhabdomyolysis [7]. It aims to distinguish the patients in whom the external triggers are sufficient to explain the episode of rhabdomyolysis from those in whom the external triggers insufficiently explain the (severity of the) rhabdomyolysis event.

So far, a number of Mendelian genetic defects have been identified to increase rhabdomyolysis susceptibility, including a number of genes implicated in muscle metabolism and mitochondrial function (e.g., *ACADVL*, *CPT2*, *PYGM* or *LPIN1*), [8–10,11] and various muscular dystrophies (e.g., Becker muscular dystrophy and limb girdle muscle dystrophy 2I [LGMD2I]) [12], or in certain congenital myopathies with specific defects in calcium homeostasis and

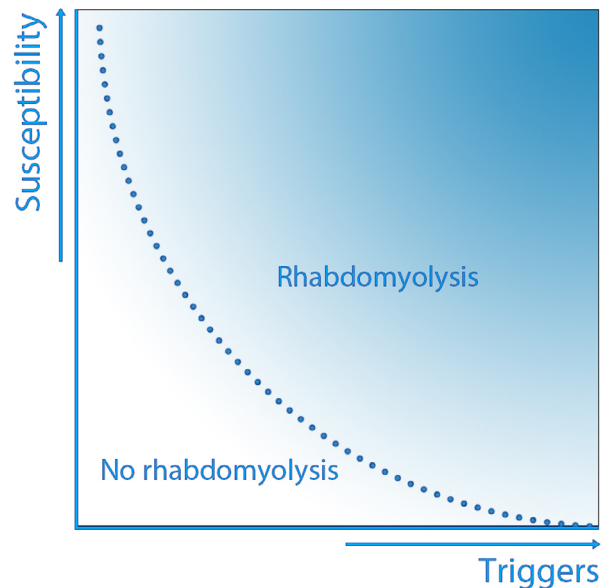


Figure 1 Graph showing that rhabdomyolysis events can be attributed to a combination of environmental factors (e.g., strenuous exercise and/or febrile infection) and a predisposing genotype. A combination of external triggers and genetic predisposition may thus be required for an individual to exceed the threshold for developing rhabdomyolysis. [Colour figure can be viewed at wileyonlinelibrary.com]

excitation-contraction coupling (e.g., *RYR1*) [13]. Such marked genetic heterogeneity poses a considerable diagnostic challenge for clinicians [5]. The awareness of specific genotype–phenotype correlations is of great importance, not only for establishing the correct genetic diagnosis, but also for effective personalized counseling. The means to detect a genetic contribution have increased markedly since the introduction of next-generation sequencing; therefore, an observational study on genetic variants implicated in rhabdomyolysis susceptibility may be particularly useful.

In the present single-center study we reviewed all patients with a serum CK level of ≥ 2000 IU/l, admitted or referred to our center between January 2014 and January 2019. First, we described the external triggers and epidemiological data in all patients. Next, those with a proven or presumed genetic susceptibility were further assessed by reviewing patient data records, resulting in an overview of all genetic variants and clinical characteristics in both subsets Box 1.

Materials and methods

Identification

A flow diagram illustrating the selection process is shown in Fig. 2. Medical records of patients admitted

Box 1. A genetic defect increasing rhabdomyolysis susceptibility can be considered in cases fulfilling one or more ‘RHABDO’ criteria, described below [5]

- R - Recurrent episodes of exertional rhabdomyolysis
- H - HyperCKaemia persisting more than 8 weeks after event
- A - Accustomed physical exercise
- B - Blood creatine kinase (CK) $>50 \times$ upper limit of normal
- D - Drug ingestion/medication/supplements or other exogenous and endogenous factors cannot sufficiently explain the rhabdomyolysis severity
- O - Other family members affected / other exertional symptoms (e.g. cramps or myalgia)

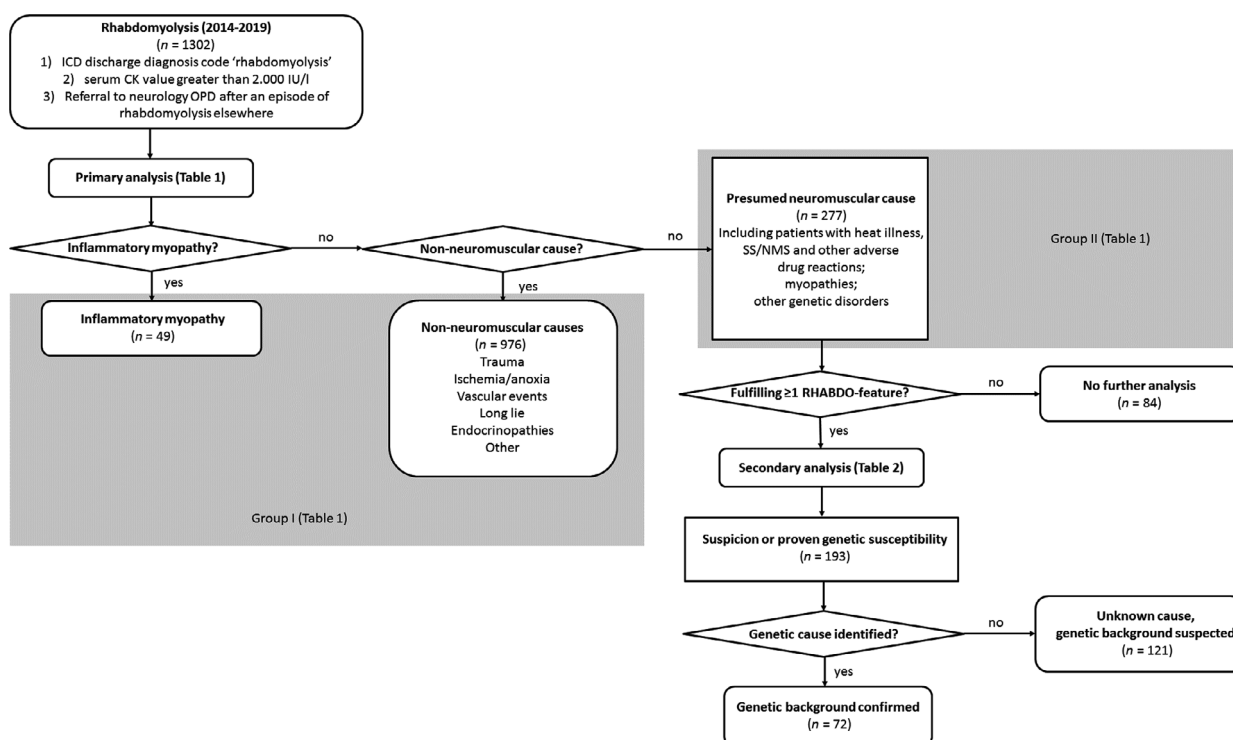


Figure 2 Flow diagram of the study selection process. CK, creatine kinase; ICD, International Classification of Diseases; NMS, neuroleptic malignant syndrome; OPD, outpatient department; SS, serotonin syndrome.

to the Radboud University Medical Center (Nijmegen, The Netherlands) between January 2014 and January 2019 were reviewed. Patients were selected if they (i) had the International Classification of Diseases discharge diagnosis code 'rhabdomyolysis' and (ii) had a serum CK value >2.000 IU/l. A recent study amongst 10 096 healthy US adults concluded that the upper limit of normal (ULN) of CK values may vary from 194 to 1001 IU/l, depending on sex, ethnicity and training levels [14]. Therefore, taking into account these baseline differences, a CK threshold of 2000 IU/l was determined, reducing the chance of including possibly healthy individuals. Lastly, patients were included if they (iii) were referred to the neurology outpatient department after an episode of rhabdomyolysis, for which they had been admitted elsewhere.

Triggers

First, for our primary analysis, triggers causing rhabdomyolysis events were analyzed and subdivided into two groups: (i) presumed neuromuscular causes and (ii) non-neuromuscular causes or persisting hyperCKaemia due to muscular dystrophies. The second group was subdivided into: ischemia/anoxia (including vascular occlusion, thrombo-embolism, shock, aortic dissections or asphyxia); traumatic muscle injury; 'long lie' (defined as

having lain on the floor or ground for over an hour after falling) [15]; postoperative status; convulsions; endocrinopathies (including electrolyte disturbances, hypo- and hyperthyroidism or diabetic decompensation); acquired myopathies (including idiopathic inflammatory myopathies); 'other, genetic' or 'other, acquired'.

The following patients were classified as having (presumed) neuromuscular disorders: those with muscle dystrophies, metabolic or mitochondrial myopathies, congenital myopathies or other inherited muscle diseases, those with heat-illness (including heat cramps, heat syncope, heat exhaustion or heat stroke); and those with adverse drug reactions, subdivided into neuroleptic malignant syndrome (NMS), serotonin syndrome (SS) or 'other adverse drug reactions'. NMS or SS was diagnosed if rhabdomyolysis was associated with exposure to medication known to cause these syndromes, and the patient had symptoms of tremor and rigidity (NMS), or if the patient fulfilled the Hunter criteria (SS) [16]. Finally, patients were classified as 'other' if they were diagnosed with another disease, not included in the above group (e.g. Prader-Willi syndrome, G6PD deficiency).

Data extraction

After identifying the triggers and underlying susceptibilities, patients in the group with a (presumed)

neuromuscular cause, fulfilling any of the RHABDO features (Box 1) were selected for our secondary analysis. These included patients with recurrent episodes of rhabdomyolysis, hyperCKaemia persisting 8 weeks after the event, CK levels >10 000 IU/l, or a positive family history of neuromuscular symptoms (including cramps, myalgia or proven neuromuscular diseases). The term 'cramps' will be used to indicate either cramps (neurogenic) or contractures (myopathic). Features 'A' (accustomed to exercise) and 'D' (drug ingestion insufficient to explain event of rhabdomyolysis) were omitted, since these data were not available through patient chart review. Information was extracted from each patient data record regarding: clinical characteristics (including sex, age, medical history, family history, a recurrent pattern of rhabdomyolysis); laboratory results (including peak CK level, hyperCKaemia lasting >8 weeks [only if CK levels were followed up]); symptoms of rhabdomyolysis (including myalgia, muscle cramp/muscle weakness/muscle stiffness, myoglobinuria or dark urine); and exposure to potentially myotoxic medication at first presentation; [17,18] presence of specific triggers preceding the rhabdomyolysis episode or contributing to worsening of symptoms (including strenuous exercise, episodes of fever/infection, exposure to heat or cold, dehydration, illicit drug use, excessive alcohol intake (>10 units alcohol per week), long lie, prescription of potentially myotoxic medication, metabolic causes (hypo- or hyperthyroidism, vitamin D deficiency), seizures, or trauma, and outcome (alive or dead).

Subsequently, data were extracted regarding the extent of genetic testing performed. Whole-exome sequencing was available in our hospital since 2013. Until then, conventional sequencing methods (e.g. Sanger sequencing) were used.

Genes in which variants were found were compared with the genes covered in a previously published review on genes associated with rhabdomyolysis [5]. For each additional potentially pathogenic variant found, a systematic search in PubMed was conducted, using the keywords 'rhabdomyolysis', 'hyperCKaemia', 'myoglobinuria' and the generic name of the gene potentially implicated, to identify any relevant literature. The detailed search strategy is outlined in Appendix 1.

Statistics

Categorical data were compared using the chi-squared test or Fisher's exact test in case of fewer than five patients. Continuous variables were compared using the Mann-Whitney *U*-test.

Results

Primary analysis

A total of 1302 patient records were selected (Table 1). Of these, 934 (72%) were male and 368 (28%) were female. The median (range) age was 53 (0–98) years. Death during the episode of increased CK level was reported in 289 patients (22%). The median (range) peak CK was 4423 (2008–5 013 800) IU/l. In all categories, male patients were affected more frequently than female, except for those in the long lie group ($P < 0.001$). Patient characteristics are described in Table 1.

Of 1302 patients, 1025 (79%) had a non-neuromuscular cause for the rhabdomyolysis event, 277 (21%) had a proven genetic susceptibility, or were suspected of having a neuromuscular condition at the time of the event (Table 1). Ischemia/anoxia was the most common trigger, followed by trauma; these two triggers were responsible for 42% and 20% of all cases, respectively.

Secondary analysis

Of the group of 277 patients with a (presumed) neuromuscular cause, 193 fulfilled at least one of the RHABDO features (Table 2) and were included in the secondary analysis. These involved 139 male (72%) and 54 female patients (28%) with a median (range) age of 31 (0–94) years. Recurrent episodes were present in 99 patients (63%) fulfilling at least one RHABDO feature, 45 patients (51%) had a CK level >1.5 times the ULN lasting longer than 8 weeks, 134 patients (69%) had a CK level of >10 000 IU/l and 45 patients (30%) had a family history of neuromuscular symptoms. Myoglobinuria was present in 54 patients (45%). In 88 patients (45.6%), multiple RHABDO features were present. Exercise was the most common trigger of rhabdomyolysis in 107 patients (55%), followed by 55 patients (28%) in whom one or more potentially myotoxic medication was prescribed during the episode, including statins (28 patients), opioids (25 patients), antipsychotic agents (12 patients) selective serotonin reuptake inhibitors (10 patients) and tricyclic antidepressants (four patients) [19]. In 92 patients (48%), multiple triggers could be identified. Death during the episode of rhabdomyolysis occurred in 17 patients (9%), and 20 patients were admitted to the intensive care unit (20%).

Seventy-two patients (37%) proved to have a pathogenic variant in a neuromuscular gene that explained the increased susceptibility to developing rhabdomyolysis. Variants were identified on genetic

Table 1 Triggers and genetic factors underlying rhabdomyolysis in 1302 patients admitted to the Radboud University Medical Center between 2014 and 2019

Category	Patients, <i>n</i> (%) (<i>N</i> = 1302)	Median (range) age, years	Male, <i>n</i> (%)	Death, <i>n</i> (%)	Mean (SD) CK, IU/l	Median (range) CK, IU/l
I: Persisting hyperCKaemia or non-neuromuscular cause for event of rhabdomyolysis (<i>n</i> = 1025)						
Ischemia/anoxia	550 (42.2)	53.0 (0–93)	400 (72.7)	167 (30.4)	7788 (19 869)	3820 (2017–313 250)
Traumatic muscle injury	258 (19.8)	42.5 (0–98)	190 (73.6)	48 (18.6)	6576 (9729)	3792 (2014–81 732)
Long lie	67 (5.2)	76 (23–98)	32 (47.8)	20 (29.8)	9690 (18 300)	5278 (2196–120 000)
Postoperative	47 (3.6)	59 (10–80)	36 (76.6)	10 (21.2)	4255 (2948)	3298 (2008–15 740)
Convulsion	26 (2.0)	40.5 (0–83)	22 (84.6)	6 (23.0)	7525 (14 017)	2944 (2118–73 850)
Endocrinopathies	12 (0.9)	49.5 (8–91)	9 (75.0)	2 (16.7)	9234 (17 885)	3930 (2817–65 890)
Idiopathic inflammatory myopathies	49 (3.8)	58 (8–85)	28 (57.1)	5 (10.2)	7360 (5587)	5456 (2091–27 100)
Other	16 (1.2)	64.5 (0–76)	10 (55.5)	7 (38.8)	3836 (1485)	3415 (2037–6833)
II: Presumed neuromuscular cause for event of rhabdomyolysis (<i>n</i> = 277)						
Presumed genetic susceptibility						
Unknown cause	152 (11.7)	41 (1–94)	106 (69.7)	17 (11.1)	30 527 (126 140)	12 281 (2046–1 500 000)
Heat illness	13 (1.0)	45 (23–75)	11 (84.6)	1 (7.7)	7765 (7973)	4825 (2238–24 950)
SS/NMS	9 (0.7)	32 (18–71)	5 (55.6)	2 (22.2)	27 406 (32 001)	9570 (4551–92 400)
Adverse drug reactions	8 (0.6)	41 (18–68)	7 (87.5)	1 (12.5)	26 362 (63 761)	3012 (2496–184 100)
Proven genetic susceptibility						
Muscle dystrophy	55 (4.2)	13 (0–53)	52 (94.5)	2 (3.6)	111 785 (12 643)	5856 (2201–74 000)
Metabolic/mitochondrial myopathy	28 (2.2)	25 (0–66)	16 (55.1)	1 (6.9)	201 090 (926 615)	9206 (2261–5 013 800)
<i>RYR1</i> related disease	7 (0.5)	25 (16–43)	6 (85.7)	0 (0.0)	159 794 (208 989)	29 114 (4000–521 500)
Other inherited conditions	5 (0.4)	28 (14–50)	4 (80.0)	0 (0.0)	19,993 (21,446)	22 000 (2859–41 116)
Total	1302 (100.0)	53 (0–98)	934 (71.7)	289 (22.2)	15 563 (146 332)	4423 (2008–5 013 800)

CK, creatine kinase; hyperCKaemia, isolated raised serum creatine kinase; NMS, neuroleptic malignant syndrome; SS, serotonin syndrome. Other: neoplasm, acute necrotizing pancreatitis, burns patients, graft vs. host disease, transplant failure, G6PD deficiency, Prader-Willi syndrome. Patients are subdivided into two main categories (corresponding to I and II in Fig. 2): events of rhabdomyolysis with a non-neuromuscular cause or persisting hyperCKemia, and rhabdomyolysis events with a presumed neuromuscular cause.

testing either before or at follow-up after the rhabdomyolysis event. In 121 patients (63%) no genetic cause was identified.

Table 2 shows a comparison of patients with a proven genetic susceptibility (*n* = 72) with those in whom this was presumed based on the RHABDO features (*n* = 121). In both groups, a male preponderance was found. The mean age in the group with proven inherited muscle disease was 20 years younger (95% confidence interval [CI] 14.921–24869; *P* < 0.001) compared to the group without known cause. Furthermore, rhabdomyolysis was more frequently recurrent (90% vs. 48%; *P* < 0.001) and CK levels less frequently exceeded 10 000 IU/l (54% vs. 79%; *P* < 0.001). CK levels were lower in patients with a proven genetic susceptibility (median 10 870 IU/l) compared to those with a presumed susceptibility (median 17 870 IU/l, *U* = 3659; *P* = 0.019). Patients with a proven susceptibility less frequently had multiple triggers (*P* = 0.05). Death was reported more often in patients with a presumed susceptibility, but this was not statistically significant (4% vs. 12%; *P* = 0.079).

Triggers that were found significantly more often in those with a presumed genetic susceptibility were exposure to heat (0 vs. 10%; *P* = 0.005), use of excessive alcohol or illicit drugs (7% vs. 35%; *P* = 0.002), long lie (0% vs. 7%; *P* = 0.001) and the prescription of possible myotoxic medication (7% vs. 22%; *P* = 0.007).

Analysis of genetic variants

In the group of patients with a proven pathogenic variant (*n* = 72, column 1, Table 2) 22 different genes were implicated, involving 56 different variants. In the group of patients suspected of having a specific genetic susceptibility (*n* = 121, column 2, Table 2), variants in 16 different genes were found, all considered to be variants of uncertain significance (Data S1; Table S1). Whole-exome sequencing was performed in 16 patients (22%) in the group of 72 patients with a proven genetic susceptibility, and in 39 (32%) of the patients with a presumed genetic susceptibility. Results of genetic testing are listed in Table 3;

Table 2 Characteristics of rhabdomyolysis events in patients with a genetically proven muscle disease and patients with a presumed genetic susceptibility, fulfilling at least one of the RHABDO features

	Proven genetic susceptibility (N = 72)			Presumed genetic susceptibility (N = 121)			Total (N = 193)			P
Sex, n (%)										
Male	56 (77.8)			83 (68.6)			139 (72.0)			0.169
Female	16 (22.2)			38 (31.4)			54 (28.0)			
Age, years										
Median (range)	19.0 (0–62)			40.0 (1–94)			31.0 (0–94)			<0.001
Mean (SD)	22.4 (15.6)			42.0 (19.2)			34.7 (20.3)			
Underlying cause, n (%)										
Neuromuscular disorder										
Muscular dystrophy	36 (50.0)			-			36 (13.5)			
Metabolic/mitochondrial	25 (34.7)			-			25 (13.0)			
<i>RYR1</i> -related	7 (9.7)			-			7 (3.6)			
Other inherited	4 (5.6)			-			4 (2.1)			
Heat illness	-			3 (2.4)			3 (1.6)			
Medication										
SS/NMS	-			4 (3.2)			4 (2.1)			
Other ADR	-			4 (3.2)			4 (2.1)			
Unknown	-			110 (88.0)			110 (57.0)			
Peak CK, IU/l										
Median (range)	10 870 (2231–5 013 800)			17 870 (2100–1 500 000)			16 201 (2100–5 013 800)			0.019
Mean (SD)	104 608 (591 992)			17 870 (140 800)			64 346 (378 094)			
RHABDO features, n (%)	Yes	No	NR	Yes	No	NR	Yes	No	NR	
Recurrent	51 (89.5)	6 (10.5)	15	48 (47.5)	53 (52.5)	20	99 (62.7)	59 (37.3)	35	<0.001
HyperCKaemia > 8 weeks	27 (87.1)	4 (12.9)	41	18 (31.0)	40 (69.0)	63	45 (50.6)	44 (49.4)	104	<0.001
Blood CK > 10 000 IU/l	39 (54.2)	33 (45.8)	-	95 (78.5)	26 (21.5)	-	134 (69.4)	59 (30.6)	-	<0.001
Other family members	29 (47.5)	32 (52.5)	11	16 (17.6)	75 (82.4)	30	45 (29.7)	107 (70.3)	41	<0.001
Number RHABDO-features, n (%)										
1	28 (38.9)			77 (63.6)			105 (54.5)			
2	22 (30.6)			33 (27.3)			55 (28.5)			
3	15 (20.8)			10 (8.3)			25 (13.0)			
4	7 (9.7)			1 (0.8)			8 (4.1)			
Triggers, n (%)										
Exercise	45 (62.5)			62 (51.2)			107 (55.4)			0.080
Fever/ infection	13 (18.1)			26 (21.5)			39 (20.2)			0.642
Heat	0			13 (10.7)			13 (6.7)			0.005
Cold	1 (1.4)			7 (5.8)			8 (4.1)			0.149
Dehydration	2 (2.8)			5 (4.1)			7 (3.6)			0.655
Illicit drug use and/or alcohol	7 (9.7)			35 (29.0)			42 (21.7)			0.002
Long lie	0			9 (7.4)			9 (4.7)			0.020
Medication	10 (13.9)			45 (37.2)			55 (28.5)			0.007
Hypothyroidism	6 (8.3)			11 (9.1)			17 (8.8)			0.911
Vitamin D deficiency	5 (6.9)			1 (0.8)			6 (3.1)			0.025
Direct trauma	1 (1.4)			1 (0.8)			2 (1.0)			1.000
No trigger found	16 (22.2)			8 (6.6)			22 (11.4)			0.005
Outcome, n (%)										
Dead	3 (4.2)			14 (11.6)			17 (8.8)			0.079
Alive	69 (95.8)			107 (88.4)			176 (91.2)			
# Triggers, n (%)										
0	14 (19.4)			8 (6.6)			22 (11.4)			
1	33 (45.8)			46 (38.0)			79 (40.9)			
2	19 (26.4)			36 (29.8)			55 (28.5)			
3	5 (7.0)			23 (19.0)			28 (14.5)			
4	1 (1.4)			8 (6.6)			9 (4.7)			
>1 trigger, n (%)										
Yes	25 (34.7)			67 (55.4)			92 (47.7)			0.005

(continued)

Table 2 (Continued)

Symptoms, <i>n</i> (%)	Proven genetic susceptibility (<i>N</i> = 72)			Presumed genetic susceptibility (<i>N</i> = 121)			Total (<i>N</i> = 193)			<i>P</i>
	Yes	No	NR	Yes	No	NR	Yes	No	NR	
Myalgia and/or muscle cramp	53 (85.5)	9 (14.5)	10	85 (85.0)	15 (15.0)	21	138 (85.2)	24 (14.8)	31	0.401
Muscle stiffness	18 (72.0)	7 (28.0)	47	8 (53.3)	7 (46.7)	106	26 (38.8)	14 (61.2)	153	0.001
Muscle weakness	39 (78.0)	11 (22.0)	22	44 (59.5)	30 (41.5)	47	83 (66.9)	41 (33.1)	69	0.047
Muscle swelling	1 (16.7)	5 (83.3)	66	14 (63.6)	8 (36.4)	99	15 (53.6)	13 (46.4)	165	0.038
Dark urine/ myoglobinuria	27 (56.3)	21 (43.7)	24	27 (38.0)	44 (62.0)	50	54 (45.3)	65 (54.7)	74	0.076
WES performed, <i>n</i> (%)										
Yes	16 (22.2)			39 (32.2)			55 (28.5)			
No	56 (77.8)			82 (67.8)			138 (71.5)			

ADR, adverse drug reaction; CK, creatine kinase; hyperCKaemia, isolated raised serum creatine kinase; NMS, neuroleptic malignant syndrome; NR, not reported; SS, serotonin syndrome; WES, whole-exome sequencing.

variants in genes that have been previously implicated in events of rhabdomyolysis in a review by Scalco *et al.* [5] are underlined. Of the 22 different genes in which variants were found, 11 genes were not reviewed by Scalco *et al.* (*AGL*, *ACADM*, *CAPN3*, *CNBP*, *DMPK*, *MAGT1*, *SCN4A*, *SGCA*, *SGCG*, *SMPD1* and *TANGO2*). However, more recently, *TANGO2* has been implicated in events of rhabdomyolysis [20]. Information regarding the variants of uncertain significance, found in the group of 121 patients suspected of having a genetic susceptibility, is listed in Table S1. A thorough systematic literature search and description of these genes is provided in the Supporting information.

Discussion

In the present single-center study, we identified 1302 patients with rhabdomyolysis. Ischemia/anoxia was the most frequent trigger, followed by trauma. Of these patients, 193 had clinical characteristics suggestive of a genetic susceptibility, and, of those, 72 patients had a confirmed variant in a (putative) rhabdomyolysis-associated gene. Pathogenic variants in 22 different genes were identified, likely to lead to increased rhabdomyolysis susceptibility. In 121 patients, a genetic susceptibility was suspected based on the RHABDO features, but no pathogenic genetic variant was identified. These involved 39 patients in whom genetic testing was performed (32%). In 19 of these patients, one or more variants of uncertain significance were identified (49%). Patients with a proven genetic susceptibility were younger than those with a presumed susceptibility. However, this observation probably reflects the presence of congenital diseases that are often diagnosed already in infancy or childhood.

The demographic distribution of our study population was similar to that observed in previous studies, in particular with regard to the male predominance, which was observed in all subsets, except for the long lie group [3,4]. This male preponderance is a feature that has been observed in previous epidemiological studies on rhabdomyolysis [4]. In a study in patients with suspected malignant hyperthermia susceptibility, *in vitro* contracture testing was more often pathological in men, compared to women. [21]. This might be reflected by a sex-dependent or hormonal variable. Regarding adverse drug reactions, men more often adhered to medication, and were more often prescribed antipsychotics [22]. In addition, a study on drug intoxication in six hospitals in the Netherlands showed that men had more frequently misused drugs (66% of all cases) [23]. A systematic review, performed in 2016, identified all cohort studies focusing on rhabdomyolysis published between January 2006 and 2016, reviewing 16 studies with epidemiological data [24]. Most studies focused on specific subsets of patients (i.e., pediatric patients, statin users, postoperative patients, military recruits, burns patients and those with drug-related rhabdomyolysis). Three retrospective studies, performed in 2005, 2009 and 2012, conducted a hospital-wide assessment of rhabdomyolysis patients [2–4]. Table 4 summarizes the results of these three previous retrospective studies and compares these with the results of the present study. Limited information regarding genetic testing was included in these previous studies, probably reflecting the minimal availability of genetic testing at the time. In 2017, Vivante *et al.* [25] employed whole-exome sequencing in 21 unrelated patients with rhabdomyolysis, identifying disease-causing variants in nine patients (43%). In our subgroup of 197 patients

Table 3 Gene–phenotype relationships found in a subset of 72 patients with a proven pathogenic variant

Patient	Gene	Encodes	Phenotype	Zygoty	Variants
1	<u>ACADVL</u>	Very long-chain	VLCAD deficiency	Heterozygous	c.589G> A (p.Val197Met)
2–6		Acyl-CoA dehydrogenase	VLCAD deficiency	NR	NR
7	<u>AGL</u>	Glycogen debranching enzyme	Glycogen storage disease type IIIa	Compound heterozygous	c.848T> C (p.Val283Ala)
8	<u>ANO5</u>	Anoctamin V	LGMD2L	Heterozygous	c.191dup (p.Asn64fs)c.1733T> C (p.Phe578Ser)
9			LGMD2L	Heterozygous	c.191dup (p.Asn64fs)c.2272C> T (p.Arg 758Cys)
10			Asymptomatic hyperCKaemia	Homozygous	c.191dup (p.Asn64fs)
11	<u>CAPN3</u>	Calpain III	LGMD2A	Heterozygous	c.509A> G (p.Tyr170Cys) c.554A> T (p.Tyr185Phe) c.2115 + 1_2115+2dup (r.sp!?)
12	<u>CNBP</u>	Zinc finger protein IX	Myotonic dystrophy type II	Heterozygous	Repeat expansion (CCTG) intron 1
13			Myotonic dystrophy type II	NR	NR
14	<u>CPT2</u>	Carnitine palmitoyltransferase II	CPT2-deficiency	Heterozygous	c.302C> T (p.Ala101Val)
15			CPT2-deficiency	Homozygous	c.338C> T (p.Ser113Leu)
				Heterozygous	c.679C> T (p.Leu227Phe)
16	<u>DMD</u>	Dystrophin	Duchenne muscular dystrophy	Hemizygous	Del 56-78
17			Becker muscular dystrophy	Hemizygous	Del(X)(p.21.1-p. ?)
18			Duchenne muscular dystrophy	Hemizygous	Del exon 45-52
19			Duchenne muscular dystrophy	Hemizygous	Del exon 10-21
20-21			Duchenne muscular dystrophy	Hemizygous	Del exon 48-54
22			Duchenne muscular dystrophy	Hemizygous	Del exon 51
23			Duchenne muscular dystrophy	Hemizygous	Del exon 48-52
24			Becker muscular dystrophy	Hemizygous	Del exon 45-47
25-27			Becker muscular dystrophy	Hemizygous	Del exon 45-48
28			Duchenne muscular dystrophy	Hemizygous	Del exon 35-43
29			Female <i>DMD</i> carrier	Hemizygous	Del exon 61-62
30			Duchenne muscular dystrophy	Hemizygous	Del exon 75
31			Becker muscular dystrophy	Hemizygous	Del exon 34
32			Duchenne muscular dystrophy	Hemizygous	Del exon 11-17
33			Duchenne muscular dystrophy	Hemizygous	Insertion exon 37 Mutation exon 16
34			Becker muscular dystrophy	Hemizygous	Del exon 45-49
35			Duchenne muscular dystrophy	Hemizygous	Split sice mutation exon 7
36			Duchenne muscular dystrophy	Hemizygous	Dup exon 46-47
37			Duchenne muscular dystrophy	Hemizygous	Del exon 49-50
38-39			Duchenne muscular dystrophy	Hemizygous	NR
40	<u>DMPK</u>	Dystrophia myotonica protein kinase	Myotonic dystrophy type I	NR	Repeat expansion CTG (>200)
41	<u>DYSF</u>	Dysferlin	LGMD2B	Homozygous	c.356T> C (p.Leu119Pro)
42-43			LGMD2B	NR	NR
44	<u>FKRP</u>	Fukutin-related protein	LGMD2I	Homozygous	c.826C> A (p.Leu276Ile)
45	<u>HADHA</u>	Mitochondrial trifunctional protein	LCHAD deficiency	NR	c.1528G> C c.2099delG
46			LCHAD deficiency	NR	NR
47	<u>MAGT1</u>	Magnesium transporter type I	XMEN-disease	Hemizygous	c.664delC (p.Met223Cysfs*40)
48	<u>ACADM</u>	Medium chain acyl-CoA dehydrogenase	MCAD deficiency	Heterozygous	c.865.G> A
49	<u>PGM1</u>	Phosphoglucomutase	Congenital glycosylation disorder type It	Heterozygous	c.988G> C (p.Gly330Arg) c.1258T> C (p.Tyr420His)c.1264 C> T (Arg422Trp)
50			Congenital glycosylation disorder type It	Heterozygous	c.419G> A (p.Gly140Asp) c.1597C> T (p.Arg533Trp)

(continued)

Table 3 (Continued)

Patient	Gene	Encodes	Phenotype	Zygoty	Variants
51	<u>LPIN</u>	Lipin I	Acute recurrent autosomal recessive myoglobinuria	Homozygous	c.1471C> T
52	<u>PYGM</u>	Glycogen phosphorylase	McArdle disease	Heterozygous	c.148C> T (p.Arg50*)c.1700A> G (p.Gln567Arg)
53-58			McArdle disease	Homozygous	c.148C> T (p.Arg50*)
59			McArdle disease	NR	NR
60	<u>RYR1</u>	Ryanodine receptor 1		Heterozygous	c.10219G> T (p.Ala3407Ser)
61				Heterozygous	c.6961A> G (p.Ile2321Val) c.14545G> A (p.Val4849Ile)
62				Heterozygous	c.14545G> A (p.Val4849Ile)
63				NR	c.6617C> T
64-65				Heterozygous	c.7300G> A (p.Gly2434Arg)
66				Heterozygous	c.7018T> C (p.Phe2340Leu)
67	<u>SCN4A</u>	Sodium channel, type IV, Alpha subunit	Congenital paramyotonia	Heterozygous	c.3917G> T (p.Gly1306Val)
68	<u>SGCA</u>	Sarcoglycan alpha	LGMD2D	NR	NR
69	<u>SGCG</u>	Sarcoglycan gamma	LGMD2C	NR	Exon 5: IVS5 + 2T>C
70	<u>SMPD1</u>	Sphingomyelin phosphodiesterase 1	Niemann-Pick disease, type A/ B	Heterozygous	NR
71	<u>TANGO2</u>	Transport and golgi organization II	Metabolic encephalomyopathy, recurrent rhabdomyolysis, cardiac arrhythmias and neurodegeneration	Homozygous	Del(22)(q11.21)
72	mtDNA		MELAS		m.3243A> G

hyperCKaemia, isolated raised serum creatine kinase; LCAD, long-chain acyl-CoA dehydrogenase LGMD, Limb girdle muscle dystrophy; MCAD, medium-chain acyl-CoA dehydrogenase; MELAS, mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes; NR, Not reported; VLCAD, very-long-chain acyl-CoA dehydrogenase; XMEN, X-linked immunodeficiency with magnesium defect, Epstein-Barr virus infection, and neoplasia. Genes that have been previously associated with rhabdomyolysis by Scalco *et al.* [5] are underlined.

Table 4 Clinical characteristics and etiologies of rhabdomyolysis in previous hospital-wide retrospective studies

(n = 193)	2019 Present study (n = 1302)	2019 Present study <i>Subgroup analysis</i> (>1 RHABDO feature)	2012 Herraes <i>et al.</i> [2] (n = 449)	2008 Linares <i>et al.</i> [3](n = 106)	2005 Melli <i>et al.</i> [4] (n = 475)
CK threshold, IU/l	>2000	>2000	>975	>5000	>975
Male, %	72	72	69	79	68
Median (range) age, years	53 (0-98)	31 (0-94)	66 ± 21 ^a	51 ± 20 ^a	47 (4-95)
Average (± SD) peak CK, IU/l	15 563 ± 146 332	64 346 ± 378 094	14 355 (± NR) IU/l	34 153 ± 76 456	168 052 (± NR)
Death, %	22.2	8.8	18.7	12	3.4
Etiological factors, %					
Ischemia/anoxia	42	0	25	17	NR
Direct muscle trauma	20	1	24	23	9
Immobility ('long lie')	5	5	17	19	4
Seizures	2	0	2	8	7
Muscle diseases	11	37	NR	NR	10
Illicit drugs/alcohol	-	22	8	28	34
Exercise	-	55	4.3	NR	0.1

CK, creatine kinase; NR, not reported.

fulfilling the RHABDO criteria, we found proven genetic variants in 37% of the patients. Vivante *et al.* [25] described the genes associated with rhabdomyolysis and, corresponding to our approach, compared

those with the genes summarized in the review by Scalco *et al.* [4] In the present study, we found an additional 11 genes possibly involved in an increased rhabdomyolysis susceptibility. A role in increased

rhabdomyolysis susceptibility is plausible, considering that most of these genes are linked to metabolic and dystrophic neuromuscular conditions with similarities to other neuromuscular conditions with an increased rhabdomyolysis risk. To provide further supportive evidence for a potentially causative role of these genes, we conducted an extensive PubMed search, which is detailed in the Supporting information. This resulted in the notable observations described below.

Disorders of glycogen metabolism

Unlike glycogen storage disease type V (McArdle's disease) and type VII, rhabdomyolysis is generally not considered a key feature in patients with glycogen storage disease type IIIa [26,27]. A patient in the present study (patient 7, Table 3) experienced multiple episodes in which CK levels exceeded 1.5 times the ULN and subsequently returned to base levels. Rhabdomyolysis in patients with *AGL* variants has been described in the literature; however, only peak CK levels were mentioned and therefore the characteristic rise and fall in CK level was not reported, making it impossible to tell whether patients were experiencing episodes of rhabdomyolysis or, alternatively, had elevated baseline CK levels [30,31].

Disorders of fatty acid metabolism

Scalco *et al.* [4] reviewed the association between rhabdomyolysis and deficiencies in long-fatty-acid oxidation (i.e., very-long-chain acyl-CoA dehydrogenase [VLCAD] and long-chain acyl-CoA dehydrogenase [LCAD] deficiencies). In addition, we observed increased CK levels in one patient with medium-chain acyl-CoA dehydrogenase (MCAD) deficiency attributable to a pathogenic variant in *ACADM*. Rhabdomyolysis in patients with MCAD deficiency has been reported in various case reports and a more extensive clinical study [25,28,29].

Metabolic and mitochondrial disorders

The association between *TANGO2* and rhabdomyolysis has been discovered more recently and therefore was not included in the review by Scalco *et al.* However, *TANGO2* has been associated with events of rhabdomyolysis in more recently published previous studies [20].

Muscular dystrophies

In addition to the genes mentioned in Scalco *et al.* that are involved in the LGMD disorders (including *ANO5*, *DYSF* and *FKRP*), we found several other genes involving rhabdomyolysis in the context of

LGMD disorders, including *CAPN3* (LGMD2A), *SGCA* (LGMD2C) and *SGCG* (LGMD2E). In addition to the patients in our cohort, a literature search identified further studies reporting variants in these genes in patients with rhabdomyolysis or myoglobinuria, suggesting that these genes indeed play a role in increased rhabdomyolysis susceptibility [32,37,38]. However, in the above studies no longitudinal data regarding CK levels were provided, making the distinction between genuine rhabdomyolysis events and permanently elevated baseline CK levels impossible.

Other causes

We also identified variants in *MAGT1* and *SMPD1*, however, no published reports could be identified implicating these genes in rhabdomyolysis previously. In addition, considering that in the present cohort only one patient each with variants in these genes was identified, a definite role of these genes remains uncertain.

To our knowledge, a comprehensive hospital-wide assessment of rhabdomyolysis patients focusing on both external triggers and genetic factors has not yet been performed. Of the few single-center studies reported, most focused on specific patient subsets (i.e., pediatrics, postoperative bariatric patients, patients in the emergency department or intensive care unit) rather than on rhabdomyolysis patients in their entirety [14]. The present study demonstrates and emphasizes the broad genetic heterogeneity that might increase susceptibility for developing rhabdomyolysis.

The present study has several limitations. Firstly, in a retrospective single-center review, some details regarding symptoms, family history or presence of environmental triggers may not be thoroughly documented, likely leading to an underestimation of those factors. Secondly, in many patients, only one CK value was reported, making it impossible to differentiate between persisting hyperCKaemia and rhabdomyolysis, considering that the latter is defined as a rise and subsequent fall in CK values. Follow-up could have taken place at a different hospital because the study setting was a tertiary referral center. For the same reasons, in many patients, it was also not possible to ascertain if elevated CK levels persisted beyond 8 weeks, one of the RHBDO criteria for increased genetic susceptibility. Also, we could have missed patients if their CK value was below our threshold at the time of measurement, but their CK values may nevertheless have been increasing. Thirdly, our study population consisted of a selection of patients identified through a laboratory search in a large academic hospital with a particular focus on muscle diseases, and therefore might not accurately reflect the

rhabdomyolysis population presenting to a less-specialized general hospital. Also, patients were included that were referred to our center for diagnostic testing, after admission elsewhere. This leads to a more comprehensive analysis, but may also have led to a selection bias, thus influencing the representativeness of our study population regarding epidemiological characteristics.

Three of the four RHABDO features were present more often in the subset of patients with a proven genetic susceptibility ($P < 0.001$); therefore, we would recommend genetic testing in rhabdomyolysis patients with at least one positive RHABDO feature, even if a trigger may seem clearly identifiable. For example, Dlamini *et al.* [13] describe a case of a father and daughter developing an episode of rhabdomyolysis during the same viral infection. Additional testing revealed a *RYR1* variant that would have been dismissed as a viral myositis without the family history suggestive of a dominantly inherited neuromuscular disorder, prompting additional genetic testing. This example can be extrapolated to other clearly identifiable triggers, relevant for many medical disciplines (e.g., fever/infection, medication, exercise). In addition, some RHABDO features are probably more predictive than others, for example, an affected first-degree relative.

The 11 additional genes identified in the present cohort that were not reported in the previous review by Scalco *et al.* emphasize the broad and probably expanding spectrum of genetic susceptibility underlying rhabdomyolysis. Further evaluation of this heterogeneity may result in specific recommendations for patients and subsequent genetic counseling of the family to prevent recurring possible life-threatening events. With the increasing availability of next-generation sequencing, we would recommend that genetic testing be performed in a diagnostic setting, after discussion with a tertiary neuromuscular center.

In conclusion, in the present single-center study, we identified 1302 patients with increased CK levels suggestive of rhabdomyolysis. Ischemia/anoxia was the most frequently reported clearly identifiable trigger (40% of all cases), and 193 patients had clinical characteristics suggestive of an underlying genetic disorder, based on RHABDO criteria. Of those, 72 patients had a proven genetic susceptibility for the episode of rhabdomyolysis. Twenty-two different genes were identified, involving 56 variants that were possibly involved in an increased susceptibility for developing rhabdomyolysis. Of those, 11 genes have been associated with rhabdomyolysis in a previously published review (*ACADVL*, *ANO5*, *CPT2*, *DMD*, *DYSF*,

FKRP, *HADHA*, *PGM1*, *LPINI*, *PYGM*, *RYR1*). In addition, 11 genes are probably implicated in increased susceptibility (including *AGL*, *CAPN3*, *CNBP*, *DMPK*, *MAGT1*, *ACADM*, *SCN4A*, *SGCA*, *SGCG*, *SMPD1*, *TANGO2*), expanding the wide genetic spectrum underlying rhabdomyolysis susceptibility.

Disclosure of conflicts of interest

The authors declare no financial or other conflicts of interest.

Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Variants of uncertain significance.

Data S1. Genes involving proven pathogenic variants.

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Appendix 1 Search strategies

AGL

- 1) Glycogen storage disease type 3[MeSH] OR
- 2) Glycogen storage disease type 3[Tiab] OR
- 3) Glycogen storage disease type III[Tiab] OR
- 4) Glycogen debranching enzyme[MeSH] OR
- 5) Glycogen debranching enzyme[Tiab] OR
- 6) Glycogen debrancher enzyme[Tiab] OR
- 7) GDE[Tiab] OR
- 8) AGL[Tiab]

MCADL

- 1) Acyl coa dehydrogenase, medium chain[MeSH] OR
- 2) medium chain acyl coa[Tiab] OR
- 3) medium chain Acyl-coa[Tiab] OR
- 4) MCACA-dehydrogenase[Tiab] OR

5) MCAD[Tiab] OR

6) ACADM[Tiab]

CAPN3

1) LGMD2A[Tiab] OR

2) Limb girdle muscular dystrophy type 2A[Tiab]
OR

3) Limb girdle muscular dystrophy 2A[Tiab] OR

4) Calpain 3[Tiab] OR

5) CANPL3[Tiab]

DMPK and CNBP

1) Myotonic dystrophy[MeSH] OR

2) Myotonic dystroph*[Tiab] OR

3) Dystrophia myotonica[Tiab] OR

4) Steinert[Tiab] OR

5) PROMM*[Tiab] OR

6) DMPK[Tiab] OR

7) CNBP[Tiab] OR

8) Myotonin-protein kinase[Tiab] OR

9) ZNF9[Tiab] OR

10) Zinc finger protein 9[Tiab]

MAGT1

1) MAGT1[Tiab] OR

2) Magnesium transporter 1[Tiab] OR

3) XMEN[Tiab]

SCN4A

1) NAV1.4 Voltage-Gated Sodium Channel[MeSH]
OR

2) NAV1.4 Voltage-Gated Sodium Channel[Tiab]
OR

3) Sodium channel alpha subunit[Tiab] OR

4) SCN4A[Tiab]

SGCA & SGCG

1) Alpha sarcoglycan*[Tiab] OR

2) LGMD2D[Tiab] OR

3) Gamma sarcoglycan*[Tiab] OR

4) LGMD2C[Tiab]

SMPD1

1) Sphingomyelin phosphodiesterase[MeSH] OR

2) Sphingomyelin phosphodiesterase[Tiab] OR

3) Sphingomyelin cholinephosphohydrolase[Tiab]
OR

4) Sphingomyelinase[Tiab]