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LETTER TO THE EDITOR

"Human Stress Syndrome" and the Expanding Spectrum of RYR1-Related Myopathies

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Dear Editor,

We read with great interest the hypothesis recently presented by Zhao et al. on the relationship between "Human Stress Syndrome (HSS)" (their summary term for exertional hyperthermia-induced subacute severe myopathies) and malignant hyperthermia (MH) due to dominant mutations in the skeletal muscle ryanodine receptor (*RYR1*) gene [1]. Our recent findings in various patients with *RYR1*-related exertional rhabdomyolysis (ER) [2, 3] support this hypothesis but in addition suggest different aspects of the phenotype we would like to share in this letter. We would also like to correct the numbering of the human *RYR1* mutation (corresponding to the *RYR1* mutation associated with porcine stress syndrome) in humans: this should be c.C1840T rather than c.C1804T.

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The HSS hypothesis, following the analogy of the Porcine Stress Syndrome, offers a rational explanation for the consistent similarities between exertional hyperthermia and rhabdomyolysis (summarized as ER) and MH symptoms. Zhao et al. present several arguments to support this. First, ER and MH have similar clinical manifestations. In MH, the *RYR1* mutations cause a hypersensitive RyR1 channel, that, when activated by volatile anesthetics or a depolarizing muscle relaxant, induces a prolonged and uncontrolled release of Ca^{2+} from the sarcoplasmic reticulum. This results in sustained skeletal muscle contraction which eventually leads to a metabolic acceleration with excessive heat and lactate production. This manifests as hyperthermia, acidosis, hypercapnia, and tachycardia, and subsequently results in muscle cell death producing hyperkalemia and myoglobinuria with a risk of acute renal failure and, ultimately, death. A similar cascade of events occurs in heat and exercise-related muscle exertion. Secondly, Zhao et al. cite a number of case reports, confirming the clinical overlap between MH and ER and the similarity of metabolic characteristics. Third, they refer to the finding of a positive in vitro contracture test (IVCT), one or the diagnostic criteria for MH, also in individuals with ER.

We would like to add to these observations our own findings in patients with exertional myalgia and/or rhabdomyolysis and in whom the underlying cause had remained elusive before the *RYR1* gene was sequenced, despite often already extensive, mainly metabolic investigations [2]. Out of the initial cohort of 39 families, in 14 families, we identified nine heterozygous *RYR1* mutations/variants, many of them recurrent and five previously associated with MH (p.Lys1393Arg; p.Gly2434Arg; p.Thr4288_Ala4290dup; p.Ala4295Val; and p.Arg4737Gln). Rhabdomyolysis was commonly triggered by exercise and heat and, less frequently, viral infections, alcohol, and drugs. In some cases,

only a combination of triggers appeared to prompt acute rhabdomyolysis, and a number of individuals had only one episode throughout life, despite an often intense exercise regime. Furthermore, most individuals in our series were normally strong (or even of superior athletic ability) and had no personal history of an MH reaction during or after anesthesia, although there was a suggestive history in the extended family in two families.

These observations highlight that not only exercise, but also various stressors may evoke a rhabdomyolysis episode, and that *RYR1*-related rhabdomyolysis and MH are probably both multifactorial events with low penetrance reflecting the outcome of an unfortunate combination of different factors. It is therefore not surprising that some of the *RYR1* mutations/variants found in these individuals have a relative high population frequency (2–3 in 10,000). This latter notion is also supported by findings in the RyR1 Tyr522Ser knock-in mouse [4], where only a combination of exercise and increased environmental temperatures trigger muscle breakdown. We concluded that *RYR1* mutations may account for a substantial proportion of patients presenting with unexplained rhabdomyolysis and/or exertional myalgia. This observation should raise awareness that MH susceptibility may be a feature in patients with exercise-induced myalgia and/or ER, and provides the basis to give important and potentially preventive life-style advice to affected individuals and their relatives [2].

Since the original publication of our series, we have now made additional important observations in the initially reported individuals and have also identified a number of additional patients with novel features: Follow-up of one of the patients in our original cohort illustrates that *RYR1*-related rhabdomyolysis may be evoked by various triggers within the same individual. This patient (I.II from Family 7, Fig. 1 in Dlamini et al. 2013 [2]) presented at the age of 14 years with myalgia and mild ER (CK 2500 U/l). Physical examination, muscle ultrasound, and CK at an interval were normal (CK 188 U/l). A muscle biopsy performed in her mother who had experienced two episodes of rhabdomyolysis (CK up to 56,900 U/l) was normal. At the age of 15, she presented with severe myalgia and cramps following a week at a sports camp. She had used no alcohol. The CK increased up to 378.900 U/l on the third day of admission. She was admitted to the ICU and given hyperhydration, sodium bicarbonate, and clonazepam. She developed mild renal failure, but she eventually fully recovered and CK normalized within 3 weeks. A muscle biopsy showed only mild unevenness of oxidative staining, which was in retrospect also recognized in the biopsy of her mother. Subsequently, the c.7300G>A (p.Gly2434Arg) *RYR1* mutation previously associated with MH was detected in her and her mother. She reduced the intensity of her exercise regime, but experienced a second episode 6 months later, with CK up to 110.200 U/l. Since then, she has further reduced her sport activities and used dantrolene 25 mg up to three times daily to reduce muscle cramps. Four less severe episodes of rhabdomyolysis (CK up to 38.860 U/l) occurred over a further follow-up period of 5 years. Triggers were not as evident as with the first episodes, but most likely included a viral infection, a stressful exam period at school, and lack of sleep.

Furthermore, we recently reported a man with two recurrent episodes of viral infection-induced rhabdomyolysis occurring at the age of 36 and 43 (CK up to 521,500, and 5700 IU/L, respectively)[3]. Fasting apparently did not trigger episodes, and he had no (family) history of MH or ER. A muscle biopsy revealed mild myopathic changes and unevenness of oxidative enzyme staining. *RYR1* sequencing revealed a novel heterozygous missense mutation, c.10219G>T; p.(Ala3407Ser), affecting a highly conserved amino acid. We concluded that *RYR1* mutations have to be considered in patients presenting with fever-induced rhabdomyolysis, particularly if recurrent, even without a personal or familial history of MHS. As such, *RYR1*-related rhabdomyolysis is an important differential diagnosis of various metabolic myopathies.

Another case with ER we have recently recognized is a now 18-year-old semi-professional male cyclist who has experienced probably two episodes of rhabdomyolysis after cycling. The first episode occurred at the age of 14, when he experienced very severe myalgia in his legs 4 days after a race, resulting in impaired walking. He recovered within 1 week and he continued cycling. When he had the same symptoms 2 years later, he visited his general practitioner and his CK was elevated up to 29,914 U/l. He was admitted to the ICU for rehydration and renal function remained normal. The muscle biopsy (including EM) showed an increase of internal nuclei but no cores. *RYR1* sequencing revealed two mutations c.6961A>G (p.(Ile2321Val)) and c.14545G>A (p.(Val4849Ile) previously associated with MH. The halothane-caffeine in vitro contracture test (IVCT) was positive, confirming malignant hyperthermia susceptibility (MHS). In between, he had participated in a number of cycling races which were both physically more strenuous and took place in much hotter environments. Other factors might have contributed to the occurrence of these episodes of rhabdomyolysis: use of NSAIDs because of mild flu-like symptoms before the race, and use of a caffeine gel as an energy booster. Most likely, the combination of triggers (viral infection, caffeine, strenuous exercise) caused the rhabdomyolysis events in this case.

Lastly, we have also recently reported the case of a 14-year-old patient, who was post mortem found to be heterozygous for the known *RYR1* MH mutation c.6502G>A; p.Val2168Met. He developed an ultimately

fatal MH episode during general anesthesia performed within days after unusual and strenuous exercise [5], emphasizing the connection between MH and ER and the probably multifactorial nature of both events.

This variety of triggers and the range of severity of ER episodes make the concept of a HSS proposed by Zhao et al. an attractive preposition. To stress the relation with *RYR1* mutations, it can be defined as a "non-anesthetic, *RYR1*-related rhabdomyolysis." It is essential to identify the *RYR1* mutations that increase the sensitivity of the ryanodine receptor (RyR1) and perform an IVCT to confirm MH susceptibility, with the ultimate aim to prevent potentially fatal ER or MH reactions in the patients and relatives at risk. Genetic analysis will have to be combined with functional testing and segregation analysis since many *RYR1* variants of uncertain significance occur whose functional consequences have not yet been documented in detail. Guidelines for secondary prevention need to be developed in more detail, particularly concerning exercise advice, the use of dantrolene as a preventive agent, and other medications that may be contra-indicated or only be taken with caution.

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