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Pancreatitis in *RYR1*-related disorders

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ABSTRACT

Mutations in *RYR1* encoding the ryanodine receptor (RyR) skeletal muscle isoform (RyR1) are a common cause of inherited neuromuscular disorders. Despite its expression in a wide range of tissues, non-skeletal muscle manifestations associated with *RYR1* mutations have only been rarely reported. Here, we report three patients with a diagnosis of Central Core Disease (CCD), King-Denborough Syndrome (KDS) and Malignant Hyperthermia Susceptibility (MHS), respectively, who in addition to their (putative) *RYR1*-related disorder also developed symptoms and signs of acute pancreatitis. In two patients, episodes were recurrent, with severe multisystem involvement and sequelae. RyR1-mediated calcium signalling plays an important role in normal pancreatic function but has also been critically implicated in the pathophysiology of acute pancreatitis, particularly in bile acid- and ethanol-induced forms. Findings from relevant animal models indicate that pancreatic damage in these conditions may be ameliorated through administration of the specific RyR1 antagonist dantrolene and other compounds modifying pancreatic metabolism including calcium signalling. These observations suggest that patients with *RYR1* gain-of-function variants may be at increased risk of developing acute pancreatitis, a condition which should therefore be considered in the health surveillance of such individuals.

1. Introduction

Mutations in the skeletal muscle ryanodine receptor *(RYR1)* gene are a common cause of inherited neuromuscular disorders and have been associated with a wide phenotypical spectrum, ranging from various early-onset congenital myopathies with often substantial weakness to induced phenotypes such as (exertional) rhabdomyolysis (ERM) and susceptibility to the anaesthesia-related complication Malignant Hyperthermia (MH) in otherwise normally strong individuals [1]. *RYR1* encodes the principal sarcoplasmic reticulum (SR) calcium release channel (RyR1) with a crucial role in excitation-contraction coupling (ECC), the process whereby an electrical neuronal impulse is translated into muscle contraction via intracellular calcium release prompting contractile filament interactions. *RYR1* mutations associated with permanent muscle weakness typically impair effective ECC, whereas those associated with ERM and MH result in an hyperexcitable RyR1 receptor and often disproportionately increased calcium release.

RyR1s have been implicated in essential calcium signalling processes in a wide range of tissues [2], yet human disease manifestations associated with RyR1 malfunction in organs other than skeletal muscle so far have received only little attention. RyR1s are widely expressed in the mammalian pancreas [3] and, through their role in intracellular calcium signalling processes, have been critically implicated in endocrine [4] and exocrine

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pancreatic function. Moreover, several animal studies suggest a critical role of RyR1-mediated calcium signalling also in the pathophysiology of (acute) pancreatitis [5–7].

Here we report three cases with principal features of a *RYR1*-related disorder and an additional history of otherwise unexplained (acute) pancreatitis, suggesting a novel clinical association of disturbed RyR1 function not related to striatal muscle.

2. Case histories

Case histories from patients are detailed below and summarized in Table 1. Patients were identified through a family conference [8] organized by the *RYR1* Foundation (www.ryr1.org), the support organization for individuals affected by *RYR1*-related disorders (Patient 2 and 3), and through the tertiary neuromuscular clinic (Patient 1) of one of the co-authors (TW).

2.1. Patient 1

This 26-year-old female had *RYR1*-related Central Core Disease (CCD) and Malignant Hyperthermia Susceptibility (MHS) due to a heterozygous dominant *RYR1* mutation, c.14818G>*A*; p.Ala4940Thr, previously associated with both CCD and MHS [9–11].

Aged 20 she had presented to her local hospital with complaints of abdominal pain and chest pain radiating to the back, nausea and anorexia, an altered mental state and faecal incontinence. She was found to be hypotensive on examination. Laboratory investigations including a raised CRP (18 mg/l) and raised serum amylase levels (334 IU/l) combined with the clinical picture suggested a diagnosis of acute pancreatitis. There was no history of gallstones, alcohol excess or other predisposing factors. Magnetic resonance cholangiopancreatography (MRCP) did not show any gallstones or anatomical abnormalities of the pancreas. The patient stabilized on treatment with intravenous fluids and morphine analgesia and was discharged five days after admission.

Five days following discharge, she was readmitted with abdominal pain in the right upper quadrant. Her serum amylase levels had normalized to 81 IU/l, but an abdominal CT showed diffuse pancreatic parenchymal enlargement, consistent with interstitial oedematous acute pancreatitis [12]. Measurements of serum IgG4 levels to investigate autoimmune pancreatitis [13] as well as other metabolic and laboratory investigations were negative, and the pancreatitis was considered to be idiopathic.

2.2. Patient 2

This 57-year-old female had a diagnosis of *RYR1*-related King-Denborough Syndrome (KDS) and MHS due to a heterozygous dominant *RYR1* mutation (c.7354C>T; p.Arg2452Trp), a common MHS-associated variant (www.emhg.org). Other medical problems included a history of hypertension, coronary artery disease requiring percutaneous coronary intervention, epilepsy, and non-alcoholic steatohepatitis. She had also previously undergone a hysterectomy and an appendectomy.

The first episode of acute pancreatitis occurred at the age of 46 years without a clear underlying cause identified; an MRCP at the time did not show any gallstones or pancreatic abnormalities. There was no history of obesity, diabetes mellitus, excess intake of alcohol or of medications known to trigger pancreatitis.

At the age of 54 years, she presented to the local hospital again with a six-month history of increasing epigastric pain, weight loss, nausea, vomiting and dysphagia of more recent onset. On examination, she had epigastric and umbilical tenderness, was moderately hypotensive, and had low oxygen saturations. Serum lipase levels were elevated, and an abdominal CT (Figure

Table 1
Key clinical, laboratory and imaging findings from the 3 patients presented in this paper.

	Patient 1	Patient 2	Patient 3
Genetic results	*RYR1* c.14818G>*A*; p.Ala4940Thr	*RYR1* c.7354C>T; p.Arg2452Trp	Common *RYR1* mutations excluded; positive CHCT
Clinical findings	Presentation Abdominal pain radiating to the back worse on inspiration, nausea, anorexia and altered mental state. Examination BP 104/78 mmHg, later low of 87/45	Presentation Increasing epigastric pain, nausea and vomiting, anorexia and weight loss. Examination BP 94/52, SpO_2 – 91% Epigastric and umbilical tenderness.	Presentation 12 hour history of severe epigastric pain radiating to the back, nausea and vomiting. Examination Visibly unwell with mild abdominal distension and severe epigastric tenderness; HR 120 bpm. Progression Repeated episodes of SIRS and sepsis.
Laboratory results	On admission CRP 18 mg/L (<1); Amylase 334 IU/L (<100); normal IgG4 levels	On admission (2ndevent) WCC 20.6 × 10^9 L (4–11) with 72% neutrophils (35–65); calcium 1.85 mmol/L (2.1–2.6), potassium 3.0 mmol/L (3.5–5.2); glucose 7.2 mmol/L (4–7); elevated lipase and ALT with low albumin; ANA negative; IgG4 levels normal	On admission WCC 26×10^9 L (4–11); ALT 60 U/L (7–55), AST 170 IU/L (8–48); lipase – 11,000 U/L (<1000) Subsequently Repeatedly elevated bilirubin and ALP; repeated WCC spikes; cultures from aspirated parapancreatic fluid and bronchoalveolar lavage growing *C.difficile* and *Pseudomonas/Klebsiella,* respectively.
Imaging findings	Presentation Normal MRCP CT showing diffuse parenchymal pancreatic enlargement.	Presentation MRCP Normal Subsequently CT – multiple pancreatic pseudocysts and left retroperitoneal inflammation. MRCP - Small haemorrhagic cyst and retroperitoneal fluid CT five months later – Intrapancreatic haemorrhage, coeliac artery pseudocyst.	Presentation CT scan - generalised oedema pronounced in the pancreatic head. Subsequently CT – Severe necrosis throughout entire pancreas and bilateral pleural effusions (week 1). Further CTs – Repeatedly showed intraabdominal fluid collections: in the lesser sac (week 3), adjacent to the pancreas (week 4) and at the head of the pancreas (week 5) causing CBD obstruction, as well as single recurrence of a large left pleural effusion.

ALT = alanine transaminase; ANA = anti-nuclear antibodies; AST = aspartate aminotransferase; BP = blood pressure; CBD = common bile duct; CRP = C-reactive protein; CT = computed tomography; HR = heart rate; MRCP = magnetic resonance cholangiopancreatography; WCC = white cell count.

2) demonstrated pancreatic and peripancreatic pseudocysts and inflammation involving the left retroperitoneum, an appearance compatible with pancreatitis. She also had abnormal liver function, an elevated white cell count (WCC), hypocalcaemia, hypokalaemia, hypoalbuminemia, and hyperglycaemia. An MRCP demonstrated a small haemorrhagic cyst and retroperitoneal fluid consistent with recent pancreatitis but no evidence of gallstones or other pancreatic abnormalities. Laboratory investigations did not provide any evidence for autoimmune, electrolyte or lipid abnormalities. On conservative treatment with IV fluids, antibiotics and analgesics, the patient subsequently stabilized, and her laboratory markers normalized. She was also commenced on anticoagulation to treat multiple thrombi in the right subclavian and cephalic veins, identified on venous ultrasound prompted by right upper arm pain.

Five months later, the patient suffered a further episode of severe acute pancreatitis, complicated by a coeliac artery pseudoaneurysm, intra-pancreatic haemorrhage and associated pleural effusion, requiring embolization of the splenic and left gastric arteries, stenting of the coeliac artery and a thoracotomy with decortication. Endoscopic ultrasound demonstrated no evidence of pancreatic atrophy or ductal dilation but did suggest the presence of small gallstones in the gallbladder, suspected to be the cause of the most recent episode. The patient subsequently described right upper quadrant pain precipitated by ingestion of fatty foods, consistent with biliary colic, and had also become increasingly cachectic. She underwent elective laparoscopic cholecystectomy four months later resulting in symptom resolution.

2.3. Patient 3

This 42-year-old male had a positive family history of MH, with MHS confirmed by the Caffeine Halothane Contracture Test (CHCT); genetic testing for the most common *RYR1* mutations had been negative. He also suffered from exertional heat illness (EHI) and debilitating muscle spasms, both recognized features of *RYR1*-related MHS.

At age 32, he was admitted to hospital with a 12-hour history of sudden onset, severe abdominal pain radiating into the back, nausea and vomiting. He was on losartan and amlodipine and had discontinued pravastatin 10 days before. He reported moderate alcohol intake (10–12 units per week) but had not consumed any alcohol prior to the event. On examination, he was unwell and tachycardic at 120 bpm. There was mild abdominal distension and severe tenderness in the epigastric region. Laboratory investigations showed a markedly increased WCC, highly-elevated lipase and deranged ALT and AST levels. An abdominal CT scan demonstrated generalized pancreatic oedema pronounced in the pancreatic head. The gallbladder was distended, with normal appearance of the common bile duct and no evidence of gallstones (also excluded on abdominal ultrasound). A diagnosis of acute idiopathic pancreatitis was made.

Over the next two months he followed a highly fluctuating clinical course: Initially managed conservatively with intravenous fluids and antibiotics, he intermittently required total parenteral nutrition and repeated periods of invasive ventilation (and eventual tracheostomy placement) due to intermittent clinical deterioration characterized by cardiorespiratory compromise and altered mental status. Repeated CT scans performed over the following weeks demonstrated extensive pancreatic necrosis and bilateral pleural effusions (week 1). There were multiple fluid collections in the lesser sac (week 3), in different locations adjacent to the pancreas (week 4) and at the pancreatic head compressing the common bile duct (week 5), causing obstructive jaundice. Pleural effusions and multiple peripancreatic fluid collections required repeated drainage. He had several episodes of pyrexia and neutrophilia; whilst repeatedly negative blood cultures initially prompted a suspicion of Systemic Inflammatory Response Syndrome (SIRS), subsequently positive cultures from bronchoalveolar lavage (*Pseudomonas* and *Klebsiella*) and peripancreatic fluid collections (*C.difficile*) suggested an infectious aetiology prompting repeated courses of antibiotics. Other complications involved a psychotic episode and a cardiac arrest attributed to haloperidol medication but immediately responsive to cardiac resuscitation. Due to progressive improvement, he could be weaned from the ventilator, started to ambulate and to tolerate oral feeds. His-laboratory parameters improved, including, eventually, liver function tests following ECRP-based stenting of the compressed common bile duct. Drainage from the peri–pancreatic fluid collections gradually stabilized, initially aided by octreotide prescribed to suppress pancreatic exocrine secretions.

Over the course of the following year and due to the recurrent requirement for pancreatic drains, the patient underwent a Roux-en-Y pancreaticojejunostomy [14] to allow drainage of secretions into the jejunum. He continues to suffer from chronic abdominal pain, insulin-dependant diabetes secondary to necrotising pancreatitis, localized neuropathic symptoms and medical post-traumatic stress disorder (PTSD). Common bile duct strictures required surgical bypass after four years.

3. Discussion

Here we presented three patients with clinical features of a (putative) *RYR1*-related disorder (genetically confirmed in two) and a history of otherwise unexplained (recurrent) pancreatitis without exacerbations of neuromuscular symptoms, suggesting a not previously recognized potential association. This observation has potentially important implications for the health surveillance of individuals with *RYR1*-related disease and our understanding of the pathophysiology underlying acute and recurrent pancreatitis. Although all 3 cases reported here were adults, the observed association is also likely to apply to the Paediatric *RYR1*-mutated population.

Acute pancreatitis, the leading gastrointestinal cause for hospital admissions in the United States [15], is an inflammatory syndrome of the pancreas caused by an acute injury. Globally, there are 34 cases [16] and 1.16 fatalities per 100,000 individuals. Patients are typically middle-aged or older [17], with no difference in prevalence between men and women. Acute pancreatitis has a broad spectrum of severity, from a self-limiting illness seen in 80% of patients [18] to rapidly progressing necrotising pancreatitis often associated with multisystem organ failure [18], as seen in Patient 3 in our series. Recurrent acute pancreatitis develops in 21% of patients who suffer a first episode, 36% of whom later develop chronic pancreatitis [19].

Acute pancreatitis typically presents with persistent and severe abdominal pain radiating into the back, increases in serum amylase and/or lipase level and typical features on abdominal CT; at least two of these features are required to establish the diagnosis [20]. While certain underlying causes can be identified and treated (for example removal of common bile duct stones by ERCP), treatment is largely supportive [21].

The most common causes of acute pancreatitis in the developed world are gallstones [22], found in 35–40% [23], followed closely by excess ethanol consumption, accounting for 30% of cases in the United States [22]. Other associations include congenital abnormalities (in particular pancreas divisum), laboratory abnormalities such as hypercalcaemia and hypertriglyceridaemia, neoplasms, trauma and iatrogenic factors including medications and endoscopic retrograde cholangiopancreatography (ERCP) [24].

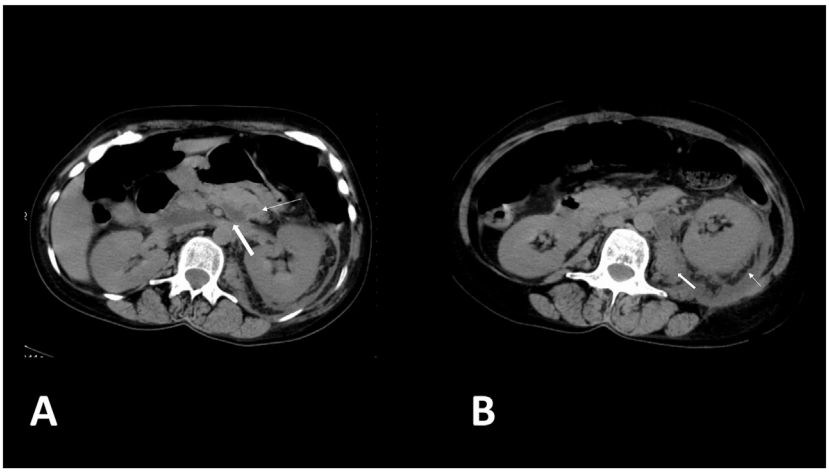

Fig. 1. Non-contrast CT of the abdomen and pelvis from Patient 2, axial sections. **A)** There is an area of low attenuation (arrow) (measuring approximately 1.1 cm x 0.9 cm) at the junction between the body and the tail of the pancreas, consistent with a pseudocyst. This low-density lesion communicates with a loculated fluid collection (another pseudocyst measuring approximately 2.7 cm x 1.7 cm) posterior to the pancreas (block arrow). Appearance consistent with sequalae of acute pancreatitis with multiple pseudocysts. **B)** Marked perinephric stranding (arrow) with multiloculated fluid collections medial to the left kidney along the anterior aspect of psoas major (block arrow). Appearance consistent with left retroperitoneal inflammation secondary to acute pancreatitis.

There are also known genetic risk factors, including inherited mutations in the cationic trypsinogen gene *PRSS1* [25], *CFTR* [26] and, less frequently, other genes such as *SPINK1*, and *CTRC* encoding chymotrypsin C [27,28]. Nevertheless, acute pancreatitis is considered idiopathic in up to 10% of patients [29] who appear to have a particularly high recurrence rate. Interestingly, mutations in *CASR* encoding the calcium sensing receptor [27,28] have also been implicated, suggesting a potential link to pancreatic calcium metabolism as also indicated by our observations.

Regardless of the causative factor, the acute insult appears to cause the failure of the safeguarding mechanisms preventing autodigestion, leading to the inappropriate intrapancreatic activation of proteases [30], particularly trypsin [31]. This initial events trigger necrosis of pancreatic parenchymal cells and a subsequent inflammatory response both locally with the intra-pancreatic recruitment of inflammatory cells [32] and systemically, leading to potentially fatal complications such as acute respiratory distress syndrome [33], SIRS (a feature in Patient 3) and subsequent multisystem organ failure [34]. Trypsinogen activation is an important early manifestation of clinical acute pancreatitis [35] and mutations predisposing to inappropriate activation have been implicated in hereditary pancreatitis [36,37]. Pathological calcium signalling, mediated through both IP3R and RyR channels, is also strongly implicated in the initiation of acute pancreatitis [38], suggesting a possible explanation for the observation reported in this study Fig. 1.

Considering that both pancreatitis and *RYR1* mutations are not uncommon in the general population, it could be argued that the association suggested in this paper is coincidental rather than causative. However, several observations, in particular the role of RyR1-mediated calcium signalling not only in normal pancreatic exocrine function but also in the pathophysiology of acute pancreatitis, suggest that such an association may indeed be biologically plausible: The role of calcium signalling in the exocrine function of pancreatic acinar cells is well-described (Fig. 2A); rises in intracellular calcium concentration are necessary for both the opening of apical membrane chloride channels [39] and subsequent fluid secretion, as well as for the exocytosis of zymogens into pancreatic secretions [40]. Functionally active ryanodine receptors (RyRs) are expressed in human pancreatic acinar cells, with limited data pointing to RyR1 as the dominant isoform [3], though RyR1 and RyR2 proteins are known to be present in rodent cells, along with RyR3 mRNA [41]. RyRs, in an intricate interplay with IP3Rs, contribute to these pancreatic calcium signalling processes under physiological conditions [42,43] and have been critically implicated in the calcium-overload state [44] that exists in pathological conditions. The distribution of the apically located IP3R [45] overlaps with the location of secretory zymogen granules, whereas RyRs [41,46] are mainly found in the basolateral region in a distribution shown by some studies to overlap with the sites of intracellular trypsinogen activation [47,5]. However, RyR-mediated release from apical acidic calcium stores may also play an important pathological role [7], and other studies have demonstrated vacuoles containing active trypsin can occur throughout the cell [48]. Stimulation of rat acinar cells by acetylcholine analogues has been shown to initiate calcium waves that, at least under physiological conditions, begin apically and spread basolaterally [49,50], a spread which is RyR-dependant and can be significantly reduced by administration of dantrolene [50]; under pathological conditions, calcium signalling is more sustained and widespread within the cell, due to mitochondrial dysfunction [51,52]. Under pathological conditions (Fig. 2B), premature activation of zymogens causing autodigestion is a key, calcium-dependent event [53] which in a secretagogue-induced model of pancreatitis has been demonstrated to occur in a compartment within acinar cells that overlaps in distribution with the RyR1 [53,5]. High-amplitude, globalised and sustained rises in intracellular calcium are critical to vacuole formation and premature zymogen activation in these cells [54], and appear to be one of the earliest pathological events observed in pancreatitis [55,56]. The two specific RyR1-mediated mechanisms that have been implicated in these processes causing acute pancreatitis, given the important protective effects of oscillatory over sustained calcium signals in acinar cells [47,39] are, i) excessive intracellular RyR-mediated calcium release [57] and/or ii) RyR-mediated calcium leak lowering the threshold of pancreatic stress required to cause pathological intracellular calcium levels. Of note in this context, the two *RYR1* variants identified in our patients were (putative) gain-of-function MH-associated variants associated with both excessive calcium release and/or calcium leak, suggesting a shift in RyR1 function that may predispose to the precise calcium imbalance also implicated in the development of pancreatitis. Potential downstream consequences of excessive RyR-mediated excessive calcium release are described in Fig. 3 and include ATP depletion by increased calcium ATPase pump activity [44] and inflammation downstream of excessive calcineurin

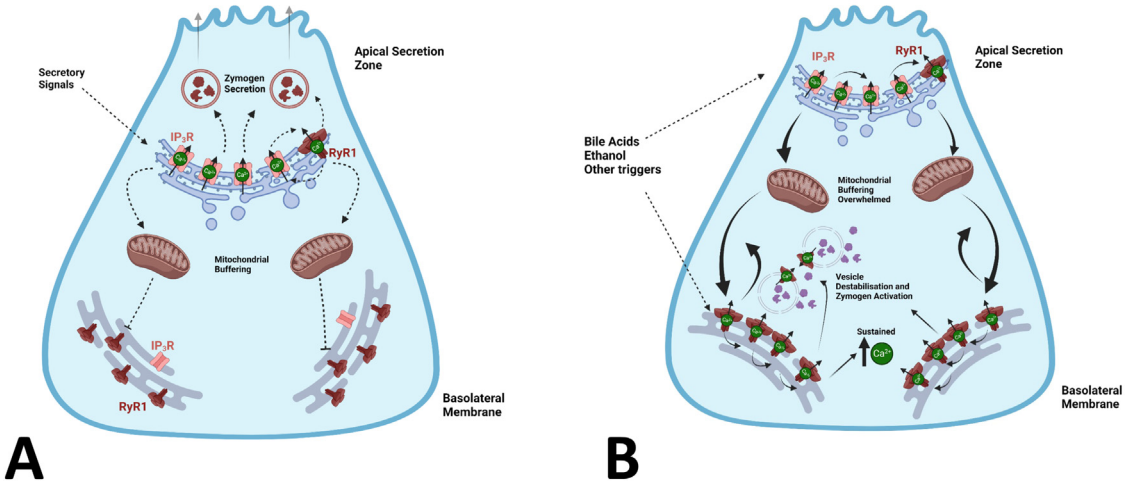

Fig. 2. Role of type 1 ryanodine receptors (RyR1) in pancreatic acinar cells under different conditions. **A)** Oscillatory calcium signals (dotted lines) originating from IP3 receptors (IP3R) activate RyR1s by calcium-induced calcium release (CICR), contributing to the rise in apical calcium that promotes zymogen secretion. Under physiological conditions, mitochondrial calcium buffering prevents the spread of calcium signals to the basolateral endoplasmic reticulum where RyR1 is predominantly expressed. Under pathological conditions **(B)**, calcium signals (bold lines) that arise apically are sustained enough to activate RyR1s in the basolateral region endoplasmic reticulum, resulting in higher intracellular calcium concentrations and, indirectly, destabilisation of zymogen-containing vesicles and premature zymogen activation. The latter processes have been shown to occur in subcellular compartments with high RyR1 expression. (Images created with Biorender; www.biorender.com).

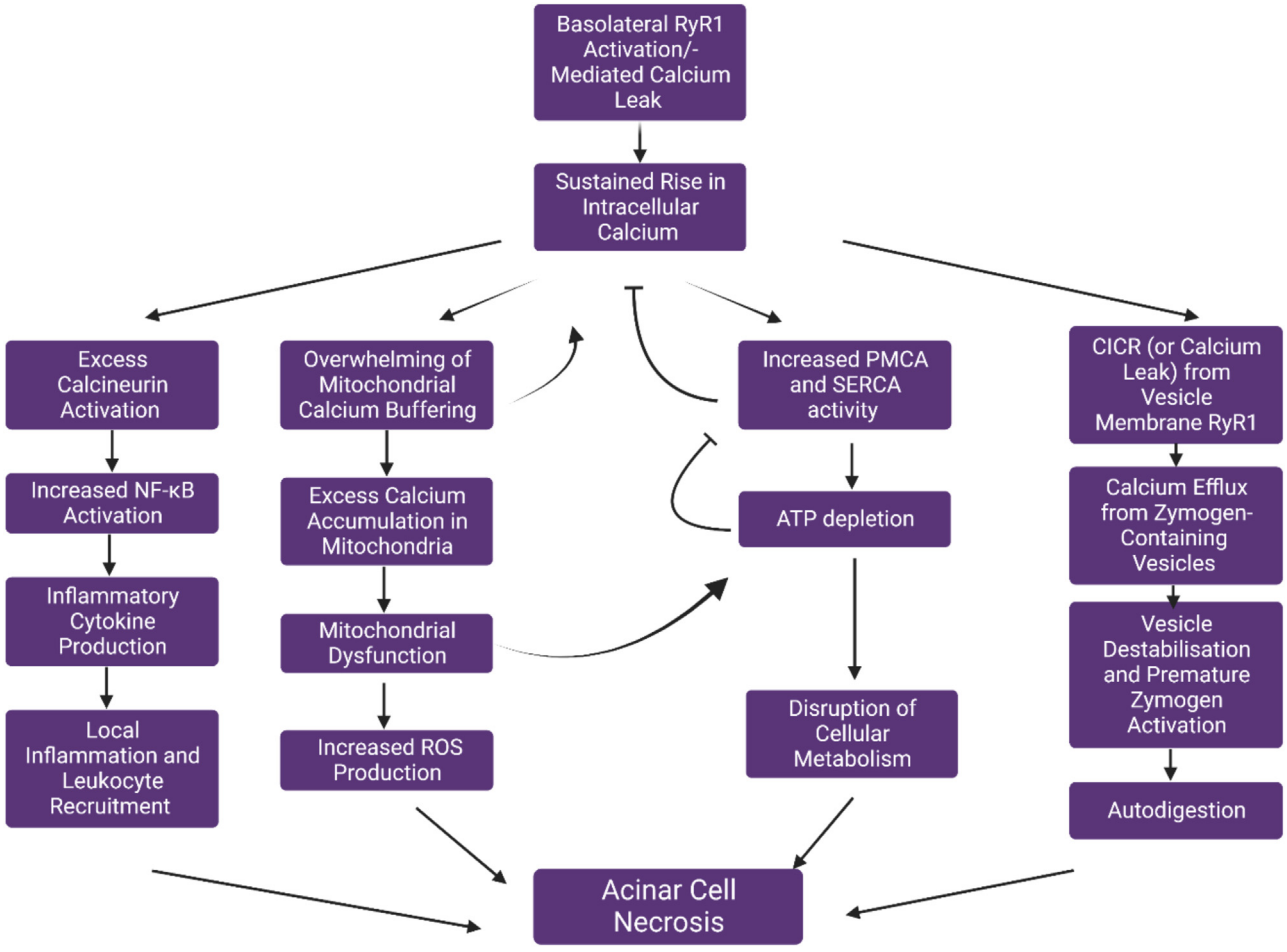

Fig. 3. Schematic representation of the consequences of RyR1-mediated pathological calcium signalling in the pancreas (selection). CICR = calcium-induced calcium release; NF-κB = Nuclear factor kappa-light-chain-enhancer of activated B cells; PMCA = plasma membrane calcium ATPase; SERCA = sarco/endoplasmic reticulum calcium ATPase; ATP = adenosine triphosphate; ROS = reactive oxygen species. (image created with Biorender; www.biorender.com).

activation [58]. Other potentially RyR-associated mechanisms that have been implicated in the pathophysiology of acute pancreatitis are iii) failure of mitochondrial calcium buffering [59–61] and iv) abnormalities of the secretory granule membranes where RyRs are also expressed [59,62–64].

In addition to their general role in idiopathic acute pancreatitis, RyRs have been implicated more specifically in the pathophysiology of the two most common forms of acute pancreatitis, gallstone- and ethanol-induced pancreatitis. Regarding the former, bile acid stimulation has been demonstrated to have various effects on acinar cell RyR1 function, including apical-to-basal propagation of calcium waves [6,65], enhanced RyR1 conductance with increased RyR1 open probability via a direct allosteric mechanism [66] and triggering of calcium release from zymogen granules [66], a process prevented by the RyR1-specific inhibitor dantrolene [67]. *In vivo* pancreatic duct obstruction in mice has been shown to cause pathological peak-plateau calcium waves in the acinar cells [68] following physiological acetylcholine stimulation; RyR1 inhibition has been shown to convert these pathological calcium waves generated by bile acid stimulation to physiological calcium oscillations and to reduce acinar cell injury [6]. Similarly to *in vivo* experiments studying secretagogue-induced pancreatitis [69], administration of dantrolene ameliorated pancreatitis in mice infused with bile acids, and even prevented pancreatic injury when given as prophylaxis [6]. Furthermore, ethanol has been shown to block apical exocytosis in rat acinar cells and cause vacuole formation in the basolateral region [70], where RyR1s are the predominant calcium channel [5]. Pre-treatment of rat pancreatic acinar cells with biologically relevant ethanol concentrations doubled intra-acinar protease activation in response to physiological secretagogue stimulation [71], in addition to doubling the speed of the apical-to-basolateral calcium waves, an RyR1-dependant process that was ameliorated by dantrolene. While the use of dantrolene likely requires further *in vivo* investigation, an alternative approach to RyR inhibition with BH4 peptides has been shown to protect isolated bile acid-exposed acinar cells from pathological calcium signalling and subsequent necrosis [72], further pointing to RyR as a valid therapeutic target.

Taken together, these observations suggest that RyR-mediated pancreatic damage plays an important role in the pathophysiology of the two most common forms of acute pancreatitis, gallstone- and ethanol-induced pancreatitis [15]. Patients with *RYR1* gain-of-function variants may be at increased risk of developing acute pancreatitis in response to common triggers, considering that the increased activity of the mutated RyR1 mimics functional changes that occur during acute pancreatitis. A similar interaction between triggering/modifying factors and genetic predisposition has already been observed with other *RYR1*-related presentations such as MH and ERM [73]. Based on findings in animal models, RyR inhibition may be an effective treatment approach not only in *RYR1*-mutated patients but also other patients with acute pancreatitis, especially if combined with treatment approaches designed to increase intracellular ATP [74]. Acute pancreatitis should be considered in the health surveillance of individuals with gain-of-function RyR1 mutations.

Declaration of Competing Interest

The authors do not have any conflicts of interest to declare in relation to this submission.

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