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Rodent models of frailty and their application in preclinical research

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ABSTRACT

In clinical medicine, the concept of frailty is viewed as a state of high vulnerability to adverse health outcomes in people of the same age. Frailty is an important challenge because the loss of physiological reserve means that even minor stressors can lead to disability and death in those who are frail. Even so, the biology of frailty is not well understood. Rodent models of frailty are stimulating research into the biology of frailty. These pre-clinical models are based on "reverse-translation". Investigators have adapted either the "frailty phenotype" approach or the "frailty index" approach, originally developed in humans, for use in animals. This review briefly describes rodent models of frailty, discusses how these models have been used to explore mechanisms of frailty and how they have been employed to assess the impact of frailty on various experimental outcomes. The review also highlights studies that have used rodent models to investigate interventions to attenuate frailty, including drug treatment, dietary modifications and exercise. The ability to model frailty in animals is an exciting development that promises to accelerate the translation of laboratory discoveries into new clinical interventions, and situates frailty research in the larger context of geroscience.

1. Introduction

It is difficult to define the health of an individual based solely on their chronological age (Rockwood et al., 2000). This heterogeneity in health during aging is present across species, including humans, dogs, rodents, and even nematodes (Kane et al., 2017; Pitt and Kaeberlein, 2015). From a clinical perspective, this presents a challenge in evaluating and managing an individual's health based simply on their chronological age. This heterogeneity is equally challenging in pre-clinical studies that investigate aging. Such studies typically use aging animals, such as rodents, to determine mechanisms of aging and responses to interventions designed to modify aging (Kõks et al., 2016). Rather than simply measuring lifespan, determining an animal's frailty provides a measure of healthspan and can indicate the efficacy of healthspan-affecting interventions (Kane et al., 2017). The recent development of rodent models of frailty reviewed here is an important new advance in the biology of aging field. The ability to use frailty as an outcome measure in preclinical aging studies can shed light on why we age and how to prevent its advancement.

1.1. The concept of frailty

While there is no internationally established definition of frailty (Bellumkonda et al., 2017; Clegg et al., 2013; Conroy, 2009; Dent et al., 2016; Morley et al., 2013; Rockwood and Mitnitski, 2011; Rodríguez-Mañas et al., 2013), it is clear that frail individuals exhibit a heightened susceptibility to adverse health outcomes when compared to non-frail individuals of the same age (Rockwood et al., 1994). Frailty is not due simply to age or multimorbidity as it is not present in all older people, occurs in younger individuals, and can occur in the absence of specific diseases (Conroy 2009; Fulop et al., 2010; Rockwood et al., 2012). The loss of physiological reserve means that even minor stressors can lead to disability and death in those who are frail (Clegg et al., 2013; Dent et al., 2016; Fulop et al., 2010; Morley et al., 2013; Rockwood et al., 2010). Proposed mechanisms of frailty include chronic inflammation, mitochondrial damage, oxidative stress, cellular senescence, endocrine dysregulation and poor DNA repair (Clegg et al., 2013; Bellumkonda et al., 2017; Fulop et al., 2010; Rockwood and Mitnitski, 2007; Walston et al., 2006; Wilson et al., 2017; Zaslavsky et al., 2013). In this, they clearly overlap with the so-called "hallmarks of aging" proposed by López-Otín et al. (2013). Still, the biology of frailty is not well understood.

1.2. Quantification of frailty in humans

Frailty is quantified in humans with many different clinical scales

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and instruments (Bouillon et al., 2013; Dent et al., 2016; de Vries et al., 2011). The two most commonly used frailty assessment tools are known as the "frailty phenotype" and the "frailty index" (FI). The frailty phenotype model was developed by Fried et al. (2001). This model conceptualizes frailty as a *syndrome* and categorises an individual's physical phenotype as robust, pre-frail or frail. Frailty is evaluated based on performance in five specific areas: unintended weight loss, exhaustion, weakness, slow walking speed, and low physical activity (Fried et al., 2001). If an individual has poor performance on three or more of these criteria they are considered frail; if they have one or two they are viewed as pre-frail and if they have none they are considered robust (Fried et al., 2001).

The FI approach was first proposed by Mitnitski et al. (2001). It conceptualizes frailty as a multidimensional *state* that arises as a consequence of the accumulation of many deficits in health. An FI is created by counting age-related deficits, such as signs, symptoms, diseases and laboratory abnormalities (Howlett et al., 2014; Mitnitski et al., 2001; Searle et al., 2008; Theou and Rockwood, 2015). The number of deficits in an individual is divided by the total number measured to produce an FI score that is theoretically between 0 (no deficits) and 1 (all possible deficits). The FI approach focuses on the number of health deficits rather than the precise nature of these deficits. It has been clearly shown that the higher the FI score the greater the chance of adverse health outcomes (Lindley, 2011; Rockwood and Mitnitski, 2011). Decades of work with this tool has revealed that FI scores have a number of signature characteristics in clinical populations. For example, deficits accumulate at a rate of ≈3% per year (Rockwood and Mitnitski, 2007), there is a limit to frailty (FI score ≈0.7) beyond which incremental deficits are not survivable (Rockwood, 2006) and the distribution of the FI broadens with age (Rockwood et al., 2004). Interestingly, women have higher FI scores than men (Gordon et al., 2017). These two different clinical frailty instruments, the frailty phenotype and the FI, are widely used in clinical studies and they do have factors in common. Both scales count the accumulation of deficits to quantify frailty and both have recently been adapted for use in rodent models, as discussed in detail below.

2. Animal models of frailty

2.1. Preclinical models based on the frailty phenotype

Aging rats and mice have been used for many decades to explore fundamental mechanisms involved in the aging process and to test interventions designed to prolong lifespan. Their relatively short life course, along with the ability to investigate the impact of age on a range of outcomes including stress responses, metabolism, behaviour and function, make rodent models invaluable for the study of aging. The growing interest in rodent models of frailty reflects the desire to understand healthspan, and not just lifespan, in the setting of aging and changes in longevity (Kane et al., 2016c). Current rodent models of frailty are summarised in Table 1, and are discussed further below.

2.1.1. Genetically manipulated mouse models of frailty in mice

The interleukin (IL)-10 knock-out mouse (IL-10$^{tm/tm}$) was originally developed as a model of inflammatory bowel disease (Rennick and Fort, 2000), although mice do not develop this disease if they are kept in strict barrier conditions (Walston et al., 2008). These mice produce no IL-10, which is an anti-inflammatory cytokine that is capable of supressing the production of pro-inflammatory cytokines (Rennick and Fort, 2000). This results in a chronic inflammatory state characterized by the increased expression of inflammatory mediators induced by nuclear factor-kappa B (Walston et al., 2008). Because chronic inflammation is implicated in the frailty phenotype (Boxer et al., 2008), Walston et al. proposed the IL-10$^{tm/tm}$ mouse as a model of frailty (Walston et al., 2008). They evaluated strength, serum IL-6 levels, activity levels, body weight and skeletal muscle gene expression in aging

Table 1
Models of Frailty in Rodents.

Basis for model	Frailty assessment tool	Species	Frailty scoring	Reference
Frailty phenotype	Knock-out the anti-inflammatory cytokine interleukin (IL)-10 to promote inflammation, weakness & low activity — model of the frailty phenotype.	Mouse	Not conducted	Walston et al. (2008)
	Knock-out the antioxidant enzyme, Cu/Zn superoxide dismutase to increase oxidative stress, promote mitochondrial dysfunction and cell senescence.	Mouse	Not conducted	Deepa et al. (2017)
	Neuromuscular healthspan scoring system to evaluate physical function & sarcopenia – model of the frailty phenotype.	Mouse	Functional assessment based on combined score from rotarod, inverted cling grip strength and *in vitro* muscle contractility.	Graber et al. (2013)
	Frailty phenotype in humans: muscle weakness, unintentional weight loss, slow walking speed, low physical activity, exhaustion (2/5 pre-frail; > 3/5 frail)	Mouse	Grip strength (weakness), weight loss, treadmill running time (exhaustion), treadmill running speed (slowness), tight rope (low activity).	Martinez de Toda et al. (2018), Gomez-Cabrera et al. (2017)
		Mouse	Modified (4 factors only): Inverted cling grip strength (weakness), rotarod (slowness), voluntary wheel running (low activity), grip test plus rotarod (exhaustion)	Liu et al. (2014)
		Rat	Modified (4 factors only): Forelimb wire suspension (weakness), rotorod (slowness), open field (low activity), inclined screen (exhaustion)	Miller et al. (2017)
Frailty index	Frailty index in humans: count the accumulation of deficits in health in an individual and divide by the total number of deficits measured. Scores can theoretically be between 0 (no deficits present) and 1 (all possible deficits present).	Mouse	Score 31 health deficits related to activity (open field), body composition (DEXA scan), metabolism (blood tests), hemodynamics (blood pressure)	Parks et al. (2012)
		Mouse	Score 31 clinical deficits in systems such as ocular, nasal, integument, musculoskeletal, digestive, urogenital, respiratory and vestibular systems.	Whitehead et al. (2014)
		Rat	Modified from Whitehead et al. (2014) and tailored for use in rats. Deficits derived from 27 clinical measures across various systems.	Yorke et al. (2017)
		Mouse	Modified from Parks et al. (2012). A 23-item laboratory frailty index was created from measures of metabolism (blood tests) blood pressure and echocardiography.	Kane et al. (2018)

female IL-10$^{tm/tm}$ mice and C57BL/6 wildtype controls. Muscle strength declined with age more in IL-10$^{tm/tm}$ mice than in controls, whereas serum levels of the pro-inflammatory cytokine IL-6 increased more in knockout mice than in wild-type (Walston et al., 2008). Skeletal muscle genes related to mitochondria and apoptosis also were differentially expressed between the two groups (Walston et al., 2008). Similar changes in strength and IL-6 have been reported in humans assessed as frail by the frailty phenotype (Boxer et al., 2008; Walston et al., 2008). On the other hand, IL-10$^{tm/tm}$ mice do not exhibit all aspects of the frailty phenotype, as there is no difference between groups in weight, activity levels or mortality up to 18 months of age (Walston et al., 2008). In addition, frailty itself has not yet been quantified in these mice (Table 1).

Another recent genetically-manipulated model of frailty is the Cu/Zn superoxide dismutase knock-out model (Sod1KO), where the antioxidant enzyme, Cu/Zn superoxide dismutase, is knocked-out (Deepa et al., 2017). These mice exhibit increased oxidative stress, mitochondrial dysfunction and cell senescence. This model was proposed because Sod1KO mice develop weight loss, weakness, low physical activity and exhaustion, which represent four of the five frailty phenotype factors (Deepa et al., 2017). They also develop inflammation plus sarcopenia and they have a shortened lifespan (Deepa et al., 2017). This is an interesting new model, but at this point relatively little is known about its characteristics. In addition, as with the IL-10$^{tm/tm}$ model, frailty has not been measured in these mice either by considering just the items specified in the phenotype or by a broader account of deficit accumulation.

2.1.2. Murine neuromuscular healthspan model

In an effort to develop a healthspan measure to assess sarcopenia in naturally-aging mice, Graber et al. (2013) proposed the neuromuscular healthspan scoring system (NMHSS; Table 1). This measure is based on the notion that sarcopenia and loss of physical function is central to the development of frailty in humans (Fried et al., 2001). Graber et al. (2013) developed a composite scoring tool based on the summed scores of a mouse's performance in rotarod and inverted-cling grip tests, plus evaluation of *in vitro* skeletal muscle contractility. Each of these functional outcomes declined with age in male C57BL/6 mice (Graber et al., 2013). In addition, extensor digitorum longus muscle force production declined with age (Graber et al., 2013). When these three individual measures were combined in a composite NMHSS score, the variability in outcome measures (*e.g.* rotarod and inverted-cling grip test) between different age groups was reduced. The authors suggest that lower variability is an advantage in evaluating the impact of interventions in aging mice (Graber et al., 2013). On the other hand, some questions about this measure remain. For example, the effect of age on the NMHSS is U-shaped, with higher scores seen at the youngest and oldest ages and lower scores seen at intermediate ages (Graber et al., 2013). The muscle contractility assessments also are invasive and limit the use of this tool in longitudinal studies.

2.1.3. Frailty phenotype models in rats and mice

Rodent frailty assessment tools based on the frailty phenotype approach used in humans (Fried et al., 2001) have recently been developed, as summarized in Table 1. Liu et al. (2014) were the first to assess the frailty phenotype in mice. They evaluated C57BL/6 mice for frailty based on most of the criteria used in people: grip strength (inverted-cling grip test), maximal walking speed (rotarod), physical activity (voluntary wheel running) and endurance (rotarod plus grip strength). A mouse is scored as non-frail, mildly frail or frail based on their performance on these tests. Scores 1.5 SD below the cohort mean are determined for each test to set the cut points. Mice with 3 or more test scores below this cut point are considered frail, 2 scores below the cut point are considered mildly frail, and fewer than 2 are considered non-frail. With this approach, they identified one mouse as frail and one as mildly frail in a sample of eleven 27–28 month old male mice (Liu et al., 2014). They argue that this frailty prevalence of 9% is similar to that in humans of a comparable age, but their sample size is relatively small and work with a larger group of mice could be informative.

Miller et al. (2017) recently adapted the original mouse frailty phenotype (Liu et al., 2014) to create a frailty phenotype instrument for use in rats (Table 1). They also used four frailty criteria (grip strength, endurance, walking speed, and physical activity) to assess frailty in 133 aged (17 mos) male Fischer 344 rats. Rats were scored as frail if they were in the lowest 20% of performance on 3 or more tests, mildly frail if they were in the lowest 20% on 2 tests and non-frail if they were in the lowest 20% on fewer than 2 tests. Results with this larger cohort showed that 23 (17%) were mildly frail and 3 (2%) were frail (Miller et al., 2017). It is important to note that the criteria used in these studies (Liu et al., 2014; Miller et al., 2017) differ from the frailty phenotype in people as they do not include a weight loss factor.

More recent work has tried to model the frailty phenotype with a weight loss factor included in the analysis. Gomez-Cabrera et al. (2017) assessed the frailty phenotype (weight loss, poor endurance, slowness, weakness and low activity levels) in groups of aging male C57BL/6 mice (17, 20, 23, 26 & 28 mos). They showed that, on average, frailty increased with age from 17 to 28 mos. However, they did not conduct all five tests in each mouse so they did not have individual frailty measures. This makes it difficult to compare their data to other studies (Gomez-Cabrera et al., 2017). Martinez de Toda et al. (2018) used this same five-factor frailty phenotype approach to quantify frailty in outbred (ICR/CD1) aging (40, 56 & 80 wks) female mice. They found that between 10 and 12% of these mice were frail. Taken together, these studies indicate that the rodent frailty phenotype is an interesting translational model that has been validated in both sexes, in different mouse strains and in different species. Important limitations of this tool include the time required to perform these tests and the requirement for specialised testing equipment.

2.2. The frailty index as applied to animal models

A promising new advance in the biology of frailty is the recent development of an FI that can be used in rodents, based on ideas first developed in people (Mitnitski et al., 2001; Rockwood and Mitnitski, 2011). These models are summarized in Table 1. Our group was the first to quantify frailty in naturally aging mice with an FI based on deficit accumulation (Parks et al., 2012). We identified 31 health-related measures based on activity levels, hemodynamic parameters, body composition and metabolism in a small cohort of male and female C57BL/6 mice (12 & 30 mos). Measures are coded as deficits based on the number of standard deviations (SD) they differ from mean reference values. Deficits that differ by more than 1 SD receive a score of 0.25, and more than 4 SD received the maximum score of 1.0. The FI score is calculated by counting deficit scores in an individual and dividing by the total number of deficits considered. This yields a continuous FI score between 0 and 1, where a higher score indicates higher frailty (Parks et al., 2012). FI scores were significantly higher in old mice than in young mice, with no difference between the sexes in this small sample (Parks et al., 2012). However, like the frailty phenotype approaches, this tool is time-consuming and requires specialised equipment. In addition, it requires invasive procedures such as blood sampling, so it is not readily utilized in longitudinal studies.

In an effort to overcome limitations of our original FI approach, we developed a mouse clinical FI tool (Whitehead et al., 2014). This approach is based on concepts developed in humans (Mitnitski et al., 2001), especially as modelled in an FI based on comprehensive geriatric assessment (FI-CGA; Jones et al., 2005). This non-invasive method uses a checklist of 31 readily observed signs of clinical deterioration (deficits) in naturally aging mice (Whitehead et al., 2014). The deficits cover a range of physiological systems such as the integument, musculoskeletal system, vestibulocochlear/auditory systems, ocular/nasal systems, digestive/urogenital systems, respiratory system and signs of discomfort (Whitehead et al., 2014). Each deficit receives a score of 0 (no

deficit), 0.5 (mild) or 1 (severe) and the scores for each deficit are added and divided by the total number measured (e.g. 31) to yield an FI score between 0 and 1. We found a graded increase in clinical FI scores from adult (5 mos), to old (19 mos) and very old (28 mos) mice (Whitehead et al., 2014). Our work has shown that key features of the FI are similar in C57BL/6 mice and people. For example, high FI scores predict mortality in mice and in humans (Rockwood et al., 2017). In addition, rates of deficit accumulation are similar in mice and in humans and the highest FI scores in mice (FI ≈ 0.55) approach the limit to frailty in humans (Whitehead et al., 2014; Rockwood et al., 2017). We also used a larger cohort of mice and found that, as in humans (Gordon et al., 2017) females have higher FI scores than males (Kane et al., 2018b). This approach is adaptable. For example, we recently modified our clinical FI tool to quantify frailty in aging rats (Yorke et al., 2017) and we have now developed an FI-Lab for use in rodents, similar to the FI-Lab used in people (Kane et al., 2018b). This simplified, non-invasive FI approach provides a powerful new tool to explore the biology of frailty, its impact on outcomes and responses to interventions designed to modify frailty.

3. Applications of frailty assessment tools

The newly developed pre-clinical models of frailty outlined in the previous section are now being used for a wide range of experimental applications. These studies are beginning to reveal new information about mechanisms involved in frailty and about the impact of frailty on different outcomes, including effects on the heart and vasculature as well as skeletal muscle and other systems. These studies are described in detail below and summarized in Table 2.

3.1. Mechanisms of frailty

Preclinical models are ideally suited to help identify mechanisms involved in the development of frailty, although relatively few studies have exploited these models to investigate such mechanisms. Walston and colleagues (2008) have used the IL-10$^{tm/tm}$ mouse to explore mechanisms of frailty. In their initial study, they report that levels of the pro-inflammatory cytokine, IL-6, are significantly higher in IL-10$^{tm/tm}$ mice of both sexes when compared to C57BL/6 controls (Walston et al., 2008). More recent work has shown that levels of a number of other pro-inflammatory cytokines, including IL-1β, tumour necrosis factor-α and interferon-γ, are elevated in serum from IL-10$^{tm/tm}$ mice compared to controls (Ko et al., 2012). Together these data support the idea that chronic inflammation plays a role in the IL-10 knockout model of frailty. Whether this is a result of frailty *per se* or is due to the lack of IL-10 in this model is not yet clear.

Our group has recently used the mouse clinical FI tool to explore links between chronic inflammation and frailty directly. We used a multiplex assay to measure 23 cytokines in serum from 17 to 23 month old C57BL/6 mice of both sexes (Kane et al., 2018b). We found that serum levels of a number of different pro-inflammatory cytokines were tightly correlated with and directly proportional to FI scores in a sex-specific fashion. For example, serum levels of IL-6, IL-9 and interferon-γ were graded by the degree of frailty in aging female mice, whereas only IL-12p40 levels rose as FI scores increased in older males (Kane et al., 2018b). These observations support the ideas that chronic inflammation plays a role in the development of frailty and that this differs between the sexes. Additional studies of links between FI scores and other putative frailty mechanisms would be of considerable interest.

3.2. Effect of frailty on outcomes

Studies in rodent models have explored the effects of age on many different physiological systems. Age itself has adverse effects on systems such as skeletal muscle (Ballak et al., 2014), the heart (Keller and Howlett, 2016) and the vasculature (Mistriotis and Andreadis, 2017); these age-dependent changes can occur even in the absence of overt disease expression. Work with pre-clinical models of frailty is revealing that frailty is a key driver of maladaptive, age-dependent remodelling in a variety of systems, as summarized in Table 2.

Most studies that have explored links between frailty and physiological function have examined effects of frailty on the heart. Parks et al. (2012) first investigated whether frailty affected the degree of age-dependent hypertrophy and contractile dysfunction in isolated ventricular myocytes from aged male and female C57BL/6 mice. They found that myocytes from old mice with high FI scores had more hypertrophy and contractile dysfunction than did age- and sex-matched mice with low scores (Parks et al., 2012). Consistent with these observations, *in vivo* echocardiography studies have shown that contractile function (*e.g.* ejection fraction) is reduced in 9 month old IL-10$^{tm/tm}$ mice when compared to wild-type mice (Sikka et al., 2013). These IL-10$^{tm/tm}$ mice also exhibit cardiac hypertrophy and slowing of cardiac relaxation when compared to controls (Sikka et al., 2013). These data suggest that this model of inflammation exhibits hypertrophy and impaired contractile function at a relatively early age, younger than is seen in naturally aging C57BL/6 mice on which the IL-10$^{tm/tm}$ model is based (Feridooni et al., 2017).

Feridooni et al. (2017) showed that the degree of left ventricular hypertrophy and contractile dysfunction in the intact heart was closely graded by the level of frailty. This study also showed that contractions and calcium transients in ventricular myocytes declined as FI scores increased (Feridooni et al., 2017). This was due to smaller calcium currents and reduced expression of the underlying calcium channel protein (Cav1.2), changes that were inversely proportional to the degree of frailty (Feridooni et al., 2017). These findings support the emerging concept that frailty arises as a consequence of the accumulation of cellular and molecular deficits that eventually scales up to produce deficits at the organ level and, ultimately at the level of the system (Howlett and Rockwood, 2013 Rockwood et al., 2015).

Links between frailty and age-related atrial dysfunction have also been reported. Moghtadaei et al. (2016) found that heart rate declined with age and that this decline was closely graded by FI score in aging mice. Sinoatrial node recovery time and conduction velocity also were prolonged with age, characteristics that were associated with increased fibrosis and alterations in the expression of matrix metalloproteinases (which regulate tissue remodelling) in the atria (Moghtadaei et al., 2016). Interestingly, these age-related changes were tightly correlated with, and graded by, FI score. Building on this work, Jansen et al. (2017) showed that these age-and frailty-dependent changes in atrial structure and function were associated with longer lasting atrial fibrillation in the mouse model. Mice with higher FI scores are also more susceptible to atrial fibrillation than mice with lower scores (Jansen et al., 2017). Together, these findings demonstrate that age and frailty dependent changes in tissue remodeling modify atrial structure and function, and thereby create a substrate for arrhythmias; these maladaptive changes are pronounced at high levels of frailty.

There is evidence that frailty modifies age-dependent deterioration in other systems (Table 2). Some of this comes from the IL-10$^{tm/tm}$ model, where stiffer blood vessels and impaired vascular relaxation have been reported as early as 9 months of age when compared to wild-type controls (Sikka et al., 2013). Older IL-10$^{tm/tm}$ mice also exhibit impaired skeletal muscle ATP kinetics (Akki et al., 2014) and mitochondrial degradation is reduced in skeletal muscle from IL-10$^{tm/tm}$ mice (Ko et al., 2016). Together, these factors may underlie the age-dependent skeletal muscle weakness that is characteristic of this model (Walston et al., 2008). The FI approach also has been used in systems other than the cardiovascular system to look at links between frailty and other outcomes. There is evidence that the mouse microbiome varies with both age and frailty, as quantified with the FI approach (Langille et al., 2014). On the other hand, there is no change in the risk of acetaminophen toxicity with either age or FI score (Kane et al., 2016a). However, there is strong evidence that high FI scores are

Table 2
Applications of frailty assessment tools.

Area of research	Frailty assessment tool	Species	Sex	Age	Study aims	Findings	Reference
Mechanisms of frailty	IL-10 knockout	Mouse	Both sexes	11 mos	Role of chronic low grade inflammation in frailty	Mouse model provides support for the idea that inflammation has a role in frailty	Walston et al. (2008), Ko et al. (2012)
	Clinical frailty index	Mouse	Both sexes	17 & 23 mos	Evaluate the links between chronic low grade inflammation in frailty	Age-dependent rise in pro-inflammatory cytokines is graded by frailty index scores. Supports a role for inflammation in frailty.	Kane et al. (2018)
Effect of frailty on bodily systems	Clinical frailty index	Mouse	Both sexes	30 mos	Links between age-dependent changes in cardiomyocytes and frailty	Maladaptive changes in cardiomyocytes more closely associated with frailty than age	Parks et al. (2012)
	IL-10 knockout	Mouse	unclear	9 mos	Role of cardiovascular dysfunction in frailty	Mouse model indicates that there is vascular stiffening and impaired cardiac function in IL-10 knockout	Sikka et al. (2013)
	IL-10 knockout	Mouse	Both sexes	22–24 mos	Role for ATP kinetics and mitochondrial degradation in skeletal muscle in frailty	Mouse model indicates that ATP kinetics are impaired and mitochondrial degradation is reduced in skeletal muscle in IL-10 knockout	Akki et al. (2014), Ko et al. (2016)
	Clinical frailty index	Mouse	Male	19–27 mos	Changing risk of acetaminophen toxicity in frailty; potential biomarkers	No increased risk of acetaminophen toxicity with frailty	Kane et al. (2016)
	Clinical frailty index	Mouse	Female	28 mos	Effect of frailty on the gut microbiome	Microbiome changes with age and frailty	Langille et al. (2014)
	IL-10 knockout	Mouse	Female	5, 12 & 22 mos	Role of changes in metabolism in frailty	Fat mass, leptin and adiponectin decline in IL-10 knockout suggesting that alterations in fat mass, hormones & energy contribute to frailty	Westbrook et al. (2017)
	Clinical frailty index	Mouse	Male	7–27 mos	Influence of frailty on cardiac ventricular function	Frailty is a key predictor of maladaptive changes in ventricular structure and function in aging mice	Feridooni et al. (2017)
	Clinical frailty index	Mouse	Male	3–24 mos	Impact of frailty on cardiac atrial function	Frailty has adverse impacts on atrial function and sinoatrial function that create a substrate for arrhythmias in aging mice.	Jansen et al. (2017) Moghtadaei et al. (2016)
	Clinical frailty index	Mouse, rat	Both	1–30 mos	Determine if frailty forecasts mortality	High frailty index scores predict mortality in wild-type mice and in 3xTg-AD mice	Rockwood et al. (2017), Yorke et al. (2017), Kane et al. (2018)

Table 3
Interventions to modify frailty in rodent models.

Intervention	Specific treatment	Frailty assessment	Age (mos)	Sex	Species	Observations	Potential clinical relevance	Reference
Drug treatment	Antioxidant (resveratrol in food; 100 mg/kg/day; 6 mos)	Clinical frailty index	18	Male	Mouse	Longevity intervention (resveratrol) delays frailty	Trial of resveratrol in humans	Kane et al. (2016)
	Polypharmacy (therapeutic doses of simvastatin, metoprolol, omeprazole, acetaminophen, and citalopram for 2 wks)	Clinical frailty index	24	Male	Mouse	Polypharmacy impairs mobility, balance and strength; no effect on frailty	Consider frailty in drug trials, deprescribing trials	Huizer-Pajkos et al. (2016)
	Sepsis (LPS, IP injection; 8 mg/kg; wait 2 wks)	8-Item frailty index	2	Male	Mouse	LPS exacerbates frailty after surgery in young mice	Pre-existing frailty may reduce resilience	Tang et al. (2017)
	mTOR inhibitor (rapamycin; 7.5–12.5 μg/day in drinking water; start at 12 mos)	Modified frailty index	24	Both sexes	Mouse	No effect of longevity intervention (rapamycin) on frailty	mTOR inhibition does not prolong healthspan	Antoch et al. (2017)
	ERß agonist (diarylpropionitrile; 3 mg/kg/day; start at 7 mos in ovariectomized mice)	Clinical frailty index	< 33	Female	Mouse	Attenuates frailty in adult mice, exacerbates it in aged	Explain complex effects of estrogen in older women	Said et al. (2018)
	ACE inhibitor (enalapril in food; 30 mg/kg/day; longitudinal; < 9 mos)	Clinical frailty index	13-25	Both sexes	Mouse	Enalapril delayed onset of frailty, especially females	Trial of enalapril in humans	Keller et al. (2018)
Exercise	Aerobic exercise (voluntary wheel running; 4 wks)	Frailty phenotype	28	Male	Mouse	Aerobic exercise reversed frailty in aging	Trial of aerobic exercise in humans	Graber et al. (2015)
	Aerobic exercise (voluntary wheel running; start at 3 mos, exercise for life)	Frailty phenotype	17–28	Male	Mouse	Long term voluntary aerobic exercise retards development of frailty	Promote lifelong exercise in humans	Gomez-Cabrera et al. (2017)
	High intensity interval training (uphill treadmill 3X/wk; 16 wks)	Frailty phenotype	24	Male	Mouse	Marked reduction of frailty in aging	Trial of high intensity exercise in humans	Seldeen et al. (2018)
Dietary modifications	Calorie restriction (40% of *ad libitium* food starting at 6 mos)	Clinical frailty index	24	Both sexes	Mouse	Longevity intervention (caloric restriction) delays frailty	Trial of calorie restriction in humans	Kane et al. (2016)
	High fat diet (33% lard in food; start at 12 mos)	Modified frailty index	18	Both sexes	Mouse	High fat diet increases frailty, reduces lifespan (males only)	High fat diet reduces resilience in males	Antoch et al. (2017)
	Calorie restriction (60% of *ad libitium food* starting at 6 mos)	Frailty phenotype	6–20	Male	Rat	Longevity intervention (caloric restriction) delays frailty	Trial of calorie restriction in humans	Todorovic et al. (2018)

associated with an increase in the risk of death. This has been reported in naturally aging C57BL/6 male mice (Rockwood et al., 2017), naturally aging Fischer 344 male rats (Yorke et al., 2017) and in female 3xTg-AD mice, which are a model of Alzheimer's disease (Kane et al., 2018a). Additional studies that consider both sexes at the same time are needed.

4. Use of frailty assessment tools to evaluate the efficacy of interventions

One promising use of the new pre-clinical models of frailty is to test novel interventions designed to modify frailty. These studies have begun to explore the impact of potentially beneficial or detrimental pharmaceutical treatments on the development of frailty. In addition, many laboratories have investigated the influence of exercise and of dietary modifications on frailty in rodent models. This work is discussed below and relevant studies are summarized in Table 3.

4.1. Impact of drug treatment on frailty in rodent models

Kane et al. (2016b) were the first to utilize preclinical models to investigate the beneficial effects of drug treatment on frailty. Resveratrol was selected as the initial pharmaceutical intervention because it is known to prolong lifespan in many models (Bhullar and Hubbard, 2015), at least in part by sirtuin-1 dependent pathways, mTOR (mammalian or mechanistic target of rapamycin) inhibition, and inducing autophagy (Howitz et al., 2003; Price et al., 2012; Park et al., 2016). Kane et al. (2016b) used the mouse clinical FI tool to quantify frailty in male C57BL/6 mice fed either standard diet or diet containing resveratrol (100 mg/kg/day) from 18 to 24 months of age. There, resveratrol treatment was associated with a significant reduction in FI scores compared to age-matched controls (Kane et al., 2016b). By contrast, 6 months of treatment with the mTOR inhibitor, rapamycin (7.5–12.5 μg/day, in drinking water), had no effect on FI scores in 24 month old male and female NIH Swiss mice (Antoch et al., 2017). Reasons for these differing results are unclear, although Antoch et al. (2017) used a different mouse strain and a modified, abbreviated FI when compared to Kane et al. (2016b). In general, FI measures that consider more deficits are more informative than FIs that have fewer items (Rutenberg et al., 2018).

A 2018 study explored the impact of chronic angiotensin converting enzyme (ACE) inhibitor treatment on frailty, quantified with the clinical FI tool, in naturally aging mice (Keller et al., 2018). ACE inhibitors are antihypertensive agents that reduce inflammation, increase skeletal muscle strength and augment muscle mass (Keller et al., 2018). Male and female C57BL/6 mice were treated with the ACE inhibitor enalapril (40 mg/kg/day in chow) for up to 9 months. FI scores were significantly lower in male and female enalapril-treated mice when treatment was started at 16 months of age (Keller et al., 2018). Interestingly, enalapril also attenuated the age-associated rise in FI scores when started at middle-age (treated from 9 to 13 months), at least in female mice (Keller et al., 2018). These beneficial effects of enalapril on frailty were mediated, in part, through sex-specific effects on inflammation. Specifically, enalapril reduced pro-inflammatory cytokines in older female mice (*e.g.* interleukin-1α, monocyte chemoattractant protein-1, macrophage inflammatory protein-1α) but increased the anti-inflammatory cytokine IL-10 in older male mice (Keller et al., 2018). These findings support the recommendation that it is critical to investigate drug effects and the underlying mechanisms in both sexes (Maric-Bilkan et al., 2016). The idea that currently approved medications such as enalapril can be re-purposed for rapid trials to improve healthspan in humans is appealing. Clinical trials of currently approved drugs, such as metformin, to treat aging and frailty are ongoing (Barzilai et al., 2016).

Reflecting common clinical scenarios (Clegg et al., 2013), rodent models of frailty are being used to investigate interventions that may exacerbate pre-existing frailty (Table 3). For example, Huizer-Pajkos et al. (2016) examined the effect of polypharmacy on frailty in C57BL/6 male mice. They administered therapeutic doses of five medications that are commonly used in older adults (*e.g.* simvastatin, metoprolol, omeprazole, acetaminophen, and citalopram, in chow) for 2 weeks and then assessed frailty with the clinical FI tool. Results show that 2 weeks exposure to polypharmacy impairs mobility, balance and strength, although it has no effect on FI scores (Huizer-Pajkos et al., 2016). As FI scores declined slightly over the two week time course of this study, it is possible that a longer exposure to the polypharmacy diet might adversely affect frailty. Additional studies would be of interest.

Other interventions to exacerbate frailty have been investigated in mice. Tang et al. (2017) induced sepsis with an IP injection of lipopolysaccharide (LPS; 8 mg/kg) and then waited two weeks to induce "pre-existing weakness". They quantified weakness with a modified physical FI that was developed based on earlier work (Whitehead et al., 2014). They then exposed mice to a stressor (surgery) and found that pre-existing weakness exacerbated frailty after surgery when compared to age-matched mice that had not been exposed to LPS (Tang et al., 2017). All the mice used in this study were young adult males, so whether this also applies to females or older animals is not yet known. Said et al. (2018) treated ovariectomized female mice with the estrogen receptor-ß agonist, diarylpropionitrile (DPN; 3 mg/kg/day) starting at 7 months of age. They had expected that DPN would attenuate frailty, as this compound has beneficial effects on systems such as mitochondrial function, metabolism and bioenergetics (Said et al., 2018). Interestingly, DPN treatment did attenuate frailty in adult mice, but exacerbated frailty in aged mice (up to 33 months). The reasons for these age-dependent effects are unclear, but the authors note that these findings are consistent with the complex effects of estrogen supplementation in older women (Said et al., 2018). This study clearly underscores that the effects of agents in younger individuals do not always predict effects in old age.

In summary, it is clear that preclinical models of frailty can be used to investigate both beneficial and detrimental treatments on the development of frailty. This provides an ideal model system to test currently approved medications for beneficial effects on healthspan, which may facilitate rapid translation to clinical trials. The idea that various stressors (*e.g.* polypharmacy, sepsis and certain drug treatments) can increase frailty in animal models is also of considerable interest. These models could be used to explore factors that may promote resilience in aging (Rockwood and Mitnitski, 2015; Kirkland et al., 2016; Schosserer et al., 2018).

4.2. Influence of exercise on frailty in rodent models

Graber et al. (2014) were the first to investigate the effects of exercise on frailty in a rodent model (Table 3). They provided aged (28–30 mos) male C57BL/6 mice with the opportunity to run on a wheel for 4 weeks and assessed frailty with the modified four factor frailty phenotype approach they had introduced earlier (Liu et al., 2014). There, aerobic exercise reversed frailty in aging male mice, even when training was started late in life (Graber et al., 2015). More recently, Gomez-Cabrera et al. (2017) used the five-factor frailty phenotype (weight loss, poor endurance, slowness, weakness and low activity levels) to extend these observations. They used 60 male C57BL/6 mice and allowed them access to a running wheel from 3 months of age to explore the impact of long term aerobic exercise on frailty. They assessed frailty at regular intervals from 17 to 28 months of age. Long term voluntary aerobic exercise retarded development of frailty in male mice (Gomez-Cabrera et al., 2017). One advantage of this work is the large sample size. On the other hand they do not have individual frailty measures for each mouse, so their information relies on pooled data, which crucially makes individualized outcome measures problematic.

Another exercise intervention that has been used to attenuate frailty in mice is high intensity interval training. Seldeen et al. (2018) used aged (24 mos) male C57BL/6 mice and assessed frailty with the five

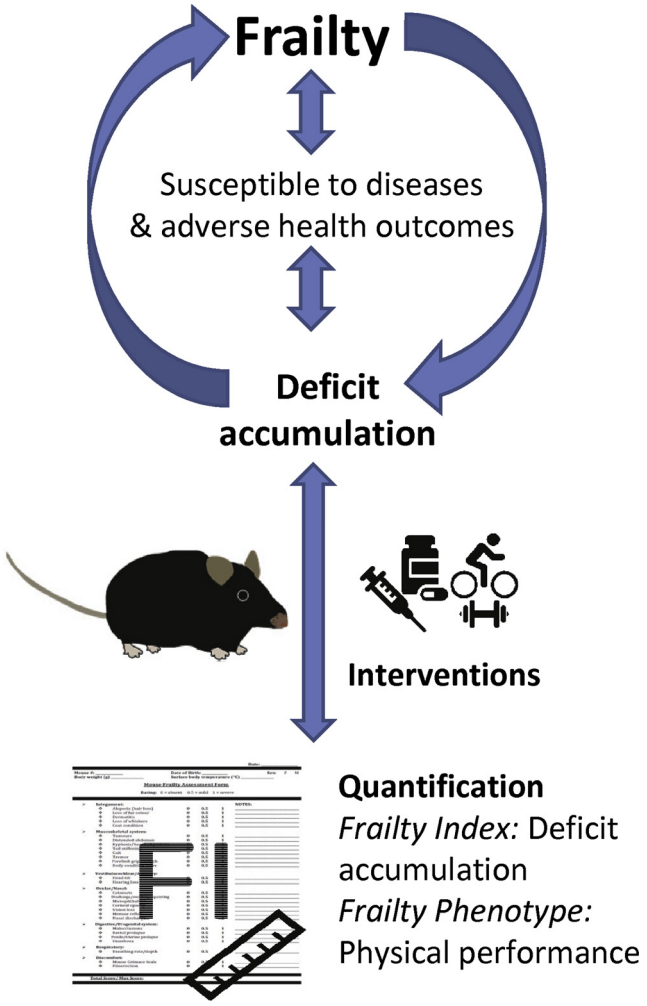

Fig. 1. Frailty as deficit accumulation in rodent models. Schematic diagram showing that frailty can be quantified as deficit accumulation with either the frailty index or frailty phenotype approach. There is a proposed negative feedback loop where disease can contribute to frailty and frailty can increase susceptibility to disease.

parameter frailty phenotype instrument. They compared frailty scores in sedentary mice with scores in mice who were subject to 10-minute uphill treadmill sessions three times per week for 16 weeks. This exercise regimen was associated with a marked reduction in the level of frailty in older male mice (Seldeen et al., 2018). These interesting findings suggest that even brief periods of increased activity may be beneficial in the setting of aging and frailty.

The results of the exercise intervention studies conducted to date provide growing evidence that both aerobic exercise and high intensity interval training can attenuate frailty. Interestingly, these exercise interventions seem to be effective even if they are started late in life. It is important to note that none of these studies used female mice, so whether exercise is equally beneficial in both sexes is not yet known. In addition, all current studies have used the frailty phenotype approach to assess frailty. It would be interesting to examine the influence of exercise on the FI in older mice, as there is evidence from human and animal studies that these two tools identify some, but not all of the same individuals as frail (Kane et al., 2017; Malmstrom et al., 2014; Hogan et al., 2012).

4.3. Dietary modifications to modify frailty in rodent models

Kane et al. (2016b) provided the initial evidence that dietary modifications can affect frailty in aging mice (Table 3). Based on strong evidence that caloric restriction can extend lifespan (Pan and Finkel, 2017), they proposed that this intervention could also prolong healthspan and attenuate frailty (Kane et al., 2016b). Kane et al. (2016b) investigated the impact of calorie restriction (40% of *ad libitium* food starting at 6 mos) in two strains of male and female mice, the short-lived DBA/2 J mice *versus* the long-lived C57BL/6 J mice. Frailty was assessed in each mouse at 18 months of age with the mouse clinical FI tool. There, calorie restriction delayed the development of frailty in both short and long lived strains of male mice (Kane et al., 2016b). Caloric restriction has no significant effect on frailty in females, although the reasons for this sex difference are not understood (Kane et al., 2016b). The impact of calorie restriction on frailty has also been explored in male rats assessed with a modified frailty phenotype approach. Todorovic et al. (2018) subjected male Wistar rats to calorie restriction (60% of *ad libitium* food starting at 6 mos). They quantified frailty at 12, 18 and 24 months, finding that calorie restriction also delayed the development of frailty in the rat model (Todorovic et al., 2018).

In addition to studies of dietary interventions designed to attenuate frailty, a dietary modification that might exacerbate frailty has been investigated. Antoch et al. (2017) placed male and female NIH Swiss mice on a high fat diet consisting of 33% lard in food, starting at 12 months of age. Frailty was assessed with a modified mouse FI tool at 18 months of age. A high fat diet augmented the level of frailty and reduced lifespan, but this was only seen in male animals (Antoch et al., 2017). The authors conclude that a high fat diet reduces resilience in males but not in females. At present it is unclear why such sex differences arise, but these results further highlight the importance of conducting research in mice of both sexes. There is now ample evidence that results from one sex cannot readily be extrapolated to the other.

5. Summary

Frailty helps explain differences in health status between individuals of the same age, with frail individuals being more susceptible to adverse health outcomes. The recognition that frailty exists in naturally aging rodent models and the adaptation of clinical tools to facilitate the quantification of frailty in these models are exciting new developments in the field. Work with these models has started to improve our understanding of frailty and its underlying mechanisms. It is becoming clear that the accumulation of deficits associated with frailty can adversely affect function in a variety of different physiological systems. This may set up a negative feedback loop, whereby frailty increases susceptibility to disease and underlying disease contributes to frailty (Fig. 1). Work with rodent models is also helping us identify interventions that can either attenuate or exacerbate frailty (Fig. 1). Ultimately these interventions may help set the stage for clinical trials to target frailty in humans.

Despite the advances outlined in this review, there are still areas that need further investigation. We are only just beginning to understand the biology of frailty and underlying mechanisms, so further work is needed. It is becoming clear that frailty adversely affects function in a number of organ systems, but how frailty may set the stage for diseases of old age is not well understood. In addition, many groups continue to use only one sex (typically male) in their studies, despite evidence that the impacts of frailty and even the underlying mechanisms might differ between sexes. There is even evidence that some interventions do not provide the same benefits in male and female animals. Other questions about interventions remain. For example, the optimal timing of frailty interventions and whether combination therapies are better than single interventions can also be readily investigated in animal models. Overall, the advent of rodent models of frailty provides us with powerful new tools to investigate these and other fundamental questions about the nature of frailty in the context of aging.

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