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Review article

ACE and ACE2: insights from *Drosophila* and implications for COVID-19

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ABSTRACT

Angiotensin-converting enzyme (ACE) and its homologue ACE2 are key regulators of the renin-angiotensin system and thereby cardiovascular function through their zinc-metallopeptidase activity on vasoactive peptides. ACE2 also serves as the receptor for the cellular entry of various coronaviruses including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the coronavirus disease 2019 (COVID-19). The unprecedented scale of the COVID-19 pandemic has spurred the use of mammalian models to investigate the SARS-ACE2 relationship and knowledge gained from such research has accelerated development of vaccines and therapeutics. Recent studies have just started to underscore the utility of the fruit fly *Drosophila melanogaster* as a model system to study virus-host interactions and pathogenicity. Notably, the remarkable existence of catalytically functional ACE and ACE2 orthologues in *Drosophila,* discovered more than two decades ago, provides a unique opportunity for further developing this model organism to better understand COVID-19 in addition to identifying coronavirus preventative and therapeutic interventions targeting ACE2. Here, we review the studies that revealed crucial insights on the biochemistry and physiology of *Ance* and *Acer*, two out of the six *Drosophila* ACE family members with the greatest homology to human ACE and ACE2. We highlight shared *in vivo* functions outside of the renin-angiotensin system, which is not conserved in flies. Importantly, we identify knowledge gaps that can be filled by further research and outline ways that can raise *Drosophila* to a powerful model system to combat SARS-CoV-2 and its threatening vaccine-evading variants.

1. Introduction

Angiotensin-converting enzyme (ACE) is a crucial regulator of the renin-angiotensin system (RAS) and, hence, cardiovascular activity through its conversion of angiotensin I (Ang I) to the potent vasoconstrictor Ang II. ACE2 provides a counter-regulatory arm to RAS through its conversion of Ang II to the shorter vasodilatory peptide Ang-(1–7), thereby providing a cardioprotective role. Whereas ACE is the target of many widely prescribed anti-hypertensive drugs, the potential of ACE2 as a therapeutic target has only emerged recently in view of its role as a receptor for various coronaviruses including the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for the coronavirus disease 2019 (COVID-19) pandemic (Hooper et al., 2020; Lubbe et al., 2020). A significant number of studies have thus investigated the SARS-ACE2 relationship and exploited gained knowledge to accelerate development of vaccines and therapeutics. Key studies have made use of mammalian models, which is not surprising given their high degree of genetic and physiological conservation to humans (Munoz--Fontela et al., 2020). Invertebrate models such as the fruit fly *Drosophila melanogaster* have been relatively overlooked, despite key advantages including a short lifecycle, high progeny numbers, minimal ethical concerns, low maintenance costs and abundant genetic resources.

The utility of *Drosophila* as a powerful tool in our arsenal for combating present and future epidemics caused by coronaviruses has only emerged recently (Nainu et al., 2020; van de Leemput and Han, 2021). Studies have thus exploited *Drosophila* to study virus-host interactions and pathogenicity. SARS-CoV-2 proteins Nsp6, Orf6 and Orf7a were found to be toxic with flies showing reduced viability and tissue defects, including defects in the trachea (fly equivalent of the lung) and muscle weakness (Zhu et al., 2021). Notably, these phenotypic findings recapitulate those reported in COVID-19 patients, where respiratory problems, lung injury and myalgia are hallmark features (Huang et al., 2020). In another study, which is still in preprint, SARS-CoV-2 protein Orf3a was also found to be deleterious in *Drosophila* when expressed in the nervous system leading to reduced lifespan, impaired motor function, cell death and neuroinflammation (Yang et al., 2020). Fatigue and neurological problems are emerging as persistent symptoms of the post-viral syndrome, termed 'Long COVID', experienced by several

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COVID-19 recovering patients (Shu et al., 2021). It is interesting that the absolute majority (90%) of human proteins in the SARS-CoV-2 human interactome are conserved in *Drosophila* (van de Leemput and Han, 2021; Zhu et al., 2021) and the identification of chemicals that mitigate phenotypes linked to SARS-CoV-2 protein expression show the potential of flies as a powerful *in vivo* drug discovery platform (Yang et al., 2020; Zhu et al., 2021). However, most remarkable is the existence of catalytically functional ACE and ACE2 orthologues in *Drosophila*. Here we provide a timely review of classic and recent studies that reveal insights on their structure, biochemistry, substrate specificity, expression, regulation, tissue distribution and function. Importantly we ask whether additional research aimed at addressing outstanding questions is warranted to further develop *Drosophila* as an additional *in vivo* model for understanding COVID-19 and, crucially, in preparation for future coronavirus outbreaks.

2. Discovery of ACE and ACE2 orthologues in *Drosophila*

ACE and ACE2 can be regarded as evolutionarily ancient enzymes when one takes into consideration the existence of orthologues in several mammalian, insect and bacterial species (Lubbe et al., 2020). Whereas in humans the number of ACE genes appears to be limited to *ACE* and *ACE2* (excluding non-expressed pseudogene *ACE3*), in *Drosophila*, there are six ACE-like genes (Table 1). Out of these, only *Ance* and *Acer*, the sole genes whose products are predicted to have zinc-metallopeptidase activity (Coates et al., 2000), have been very well characterised. All genes encode for proteins with one active site domain, a characteristic that renders them similar to mammalian ACE2 and the germinal or testicular form of ACE (gACE or tACE) (Figure 1). The latter is one of two forms derived from the mammalian *ACE* gene through use of alternative promoters. The other form is the larger somatic ACE (sACE) containing two active site domains, likely resulting from gene duplication (Hooper et al., 2020; Lubbe et al., 2020). Interestingly, Coates et al. (2000) proposed that the proximity of the *Ance-2* and *Ance-3* genes in *Drosophila* might result in a spliced product containing a two-domain protein similar to sACE in vertebrates. Notably, Ance-3 has a hydrophobic C-terminal membrane anchor that makes this single-domain protein or its hypothetical two-domain alternative (Ance-2+Ance-3) similar in structure to ACE and ACE2, which are both anchored to the plasma membrane through their C-terminus but can be proteolytically cleaved and released into the circulation (Figure 1) (Hooper et al., 2020; Lubbe et al., 2020). The absence of a membrane anchor in Ance, Ance-2, Ance-4, Ance-5 and Acer indicates that these are soluble secreted enzymes. Ance-3 is thought to be the closest to the ancestral gene, with a number of subsequent duplications leading to the other ACE-like genes in *Drosophila* (Coates et al., 2000).

The most homologous *Drosophila* proteins to ACE are Ance and Acer, where amino acid similarity is 61% (45% identity, 48% coverage) and 58% (41% identity, 45% coverage), respectively. For ACE2, homology, expressed as similarity in amino acid sequences, extends to Ance-3 (51% similarity, 32% identity, 65% coverage) in addition to Ance (56% similarity, 36% identity, 71% coverage) and Acer (54% similarity, 35% identity, 68% coverage) (DIOPT, DRSC Integrative Ortholog Prediction Tool: https://www.flyrnai.org/diopt). *Ance* was simultaneously identified by two groups in 1995 through isolation of a cDNA clone (Tatei et al., 1995) or purification of a soluble protein from *Drosophila* embryos (Cornell et al., 1995). The acronym *Ance* rather than *Ace* was adopted considering that in *Drosophila*, the *Ace* acronym was already taken for the gene that encodes acetylcholine esterase. In advanced stage embryos, high levels of Ance were detected in the epithelial cells of the midgut and the pericardial cells in the dorsal vessel or heart (Tatei et al., 1995). Ance expression was found to increase steadily during the first half of embryogenesis and this strong expression is maintained during larval, pupal and adult stages. Little or no maternal expression is detected suggesting that expression of Ance is low or absent in the ovaries (Tatei et al., 1995). However, Ance is expressed in the testes with the protein observed in vesicular structures within spermatocytes and immature spermatids (Hurst et al., 2003). *Acer* (*Angiotensin converting enzyme-related*) was identified in 1996 through a cDNA clone from an embryonic library. During embryogenesis, expression of Acer was apparent in the developing heart (Taylor et al., 1996). In adults, Acer is strongly expressed within the fat body in both the head and abdomen (Carhan et al., 2011). The remaining four ACE-like genes including *Ance-2* and *Ance-3*, which are located adjacent to the *Ance* gene, were identified in 2000 as a result of the sequencing of the complete *D. melanogaster* genome (Coates et al., 2000). Similar to mammalian *ACE* and *ACE2* (Cole et al., 2000; Crackower et al., 2002; Jia et al., 2020; Krege et al., 1995), *Drosophila Ance* and *Acer* are not essential genes (Table 1). Homozygous *Ance* mutants generated through chemical mutagenesis were viable despite a reduction in survival to adulthood with sterility observed in adult male flies (Hurst et al., 2003). However, a recent study describing *Ance* deletion alleles showed that homozygous flies were viable, fully fertile and morphologically normal (Kim et al., 2017). *Acer* null homozygotes were reported to be adult viable and fertile, though adults experienced a disrupted circadian behaviour (Carhan et al., 2011).

3. Enzymatic properties and specificity

Expression of recombinant Ance and Acer in yeast and their eventual detection in the culture medium confirmed that both enzymes are secreted, as expected from the lack of a membrane anchor domain (Houard et al., 1998). Mimicking the cardinal activity of its mammalian orthologue ACE (Lubbe et al., 2020), *Drosophila* Ance is capable of converting Ang I to Ang II and hydrolysing the vasodilatory peptide, bradykinin (Cornell et al., 1995; Houard et al., 1998). Glycosylation is not required for its secretion and enzymatic activity; however, it improves

Table 1. Characteristics of the ACE family members in humans and *Drosophila*. The active site domain of both Acer and Ance share the typical zinc-binding HE*X*H + E (where *X* is any amino acid) consensus sequence (only partially present in Ance-3) found in both ACE and ACE2 (underlined). Enzymatic activity refers to the prediction of zinc-metallopeptidase activity based on the presence or absence of the zinc-binding motif.

Protein	Protein length (aa)	Predicted TM domain	Active site region	Essential for adult viability	Enzymatic activity
ACE	1306	Yes	HHEMGHIQYY…HEAIG (N-terminus) HHEMGHIQYF…HEAIG (C-terminus)	No	Yes
ACE2	805	Yes	HHEMGHIQYD…HEAVG	No	Yes
Acer	630	No	HHELGHIQYY…HEAVG	No	Yes
Ance	615	No	HHELGHIQYF…HEAVG	No	Yes
Ance-2	611	No	FEAQSDLQYY…SDAIG	NA	No
Ance-3	844	Yes	HHEMAHIQYF…HQAVG	NA	No
Ance-4	609	No	HGTMAELQYH…GAAIA	NA	No
Ance-5	628	No	HSHMARVYYA…EFAVG	NA	No

Abbreviations: TM, transmembrane; NA, not available.

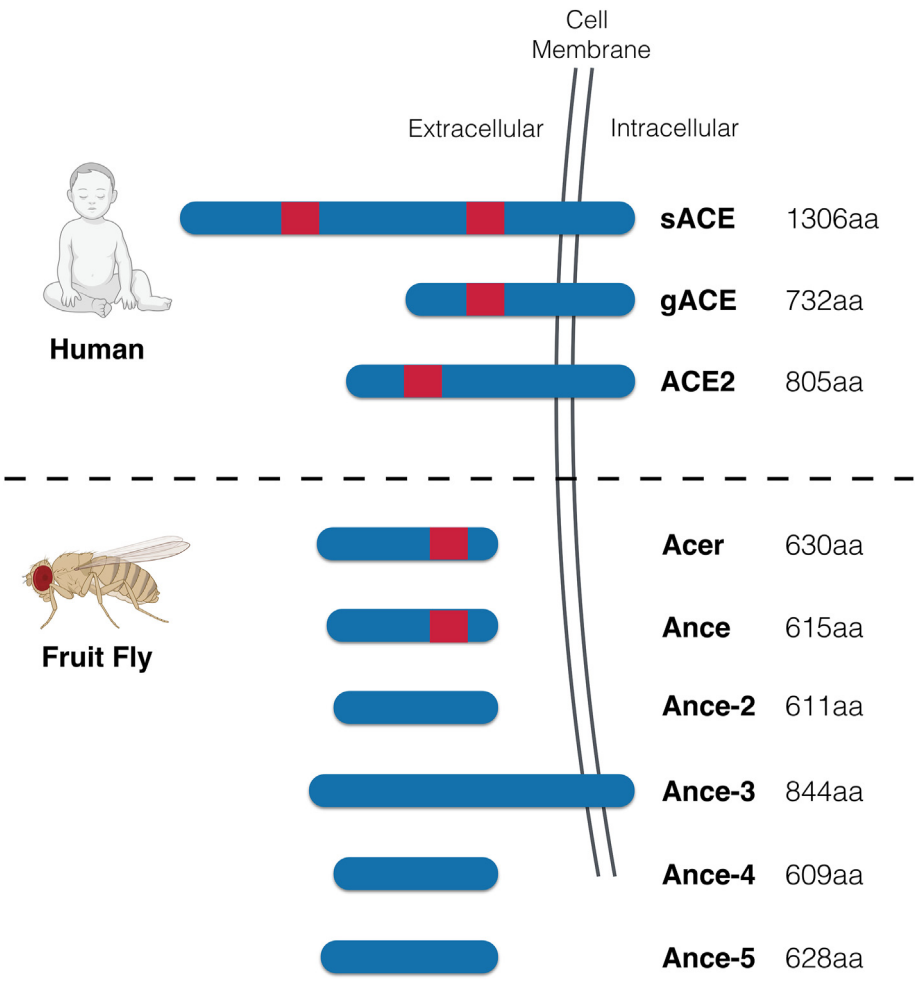

Figure 1. ACE family members in humans and fruit flies. Active site domains containing the fully conserved zinc-binding motif are indicated in red. In humans, ACE and ACE2 are integral-membrane proteins whereas in *Drosophila*, only Ance-3 is predicted to be membrane-bound. Figure created with BioRender.

protein stability (Williams et al., 1996). Although, Acer does not cleave Ang I, even at high concentrations (Houard et al., 1998), it is able to cleave bradykinin, albeit with an efficiency lower than that observed for Ance and human ACE (Bingham et al., 2006). This property makes Acer somewhat similar to ACE2 which is itself unable to cleave bradykinin (Hooper et al., 2020; Lubbe et al., 2020). Acer and Ance can also act as endopeptidases, a property also observed for mammalian ACE (Lubbe et al., 2020). Hence, both Acer and Ance were shown to cleave the C-terminal dipeptide amides from [Leu5]enkephalinamide and synthetic substrate hippuryl-L-histidyl-L-leucine-NH_2 (HHL-NH_2) as well as the C-terminal dipeptides from [Leu5]enkephalin and HHL. However, Acer hydrolyses the latter six-fold less efficiently than Ance (Houard et al., 1998). Overall, Acer displays more restricted substrate specificity compared to Ance (Siviter et al., 2002a). Both Ance and Acer can be inhibited by ACE inhibitors captopril, trandolaprilat and enalaprilat with slight variations noted, most probably the result of active site differences (Cornell et al., 1995; Houard et al., 1998).

Although the two domains of human sACE share enzymatic abilities including the ability to hydrolyse bradykinin and Ang I, they possess distinct substrate specificities (Wei et al., 1991) and differential inhibitor preferences (Dive et al., 1999; Wei et al., 1991, 1992). Furthermore, they vary in the degree to which they are activated by NaCl, with the C-domain active site being more sensitive to changes in Cl^- ion concentration (Wei et al., 1991). Ance has a strong functional resemblance to the C-domain of sACE such as the hydrolysis of substrates at comparable rates and equivalent chloride concentration requirements (Lubbe et al., 2020; Williams et al., 1996). In contrast, Acer shares structural features with the N-domain of human sACE, confirmed by the ability of N-domain selective inhibitor RXP407 to potently inhibit Acer, but not Ance. This indicates that like the sACE N-domain, the active site of Acer has a more relaxed specificity compared to that of sACE C-domain and Ance (Coates et al., 2000). Unexpectedly, Acer was also shown to be weakly inhibited by RXPA380, a highly selective inhibitor of the sACE C-domain (Bingham et al., 2006).

Structural studies of Ance bound or unbound to its ligands have revealed various insights on its structure-function relationships and those of its human orthologues (Akif et al., 2010a, 2010b, 2011, 2012; Harrison and Acharya, 2015; Masuyer et al., 2014). However, no bound Cl^- ions were recognised in the crystal structure of Ance in contrast to that observed in the structures of human ACE and ACE2 (Guy et al., 2005; Kim et al., 2003; Natesh et al., 2003, 2004), indicating that Cl^- ion binding sites in Ance are different or absent. This would explain the relatively weaker effect of NaCl on Ance's enzymatic activity (Bingham et al., 2006). Similarly, a model of the structure of Acer predicts a lack of Cl^- ion binding sites but the strong activity towards enkephalinamide peptides by NaCl suggests that Cl^- ions bind to alternative sites. Marked differences in the electrostatic charge of the substrate channel are however observed between Ance and Acer. Structural variations including different amino acids at select positions explain differences in inhibitor selectivity and potency (Bingham et al., 2006). The negative charges lining the Ance substrate channel (in contrast to the positively charged active site in Acer) favour interactions with positively charged peptide substrates. This explains the efficient cleavage of electropositive peptides such as bradykinin by Ance (Bingham et al., 2006) and, importantly, the

binding to the positively-charged receptor-binding domain (RBD) of the SARS-CoV-1 spike (S) glycoprotein to ACE2 (Prabakaran et al., 2004). Spike proteins decorate the coronavirus envelope, hence, the eponymous crown-like (corona) structure.

4. *In vivo* functions

RAS substrates are not conserved in *Drosophila* which raises questions about the *in vivo* functions of the ACE and ACE2 orthologues in this organism (Figure 2). Nonetheless, phenotypes observed on disruption of Ance and Acer are strikingly similar to those observed in mouse models. Amongst other features, ACE mouse knockouts display male infertility (Hagaman et al., 1998; Krege et al., 1995) although this is due to loss of a RAS-independent function, considering that angiotensinogen knock-out mice are themselves fertile (Kim et al., 1995). Similarly, male *Ance* mutants are sterile with testes lacking individualised sperm, indicating a role for Ance in spermatid differentiation and individualisation (Hurst et al., 2003). Ance is thus present in high concentrations in *Drosophila* testes, accumulating in vesicles in spermatocytes (Hurst et al., 2003), and in a similar manner, gACE is enriched in murine male germ cells (Sibony et al., 1994). Ance and its mammalian counterpart might have an as yet undefined but evolutionary-conserved role in the processing of peptides secreted by germ cells. A RAS-dependent function for mammalian sACE has also been proposed to occur in the prostate (Leung and Sernia, 2003; O'Mahony et al., 2005; O'Mahony et al., 2000), which is critical for the production of seminal fluid. The *Drosophila* equivalent of the prostate is the male accessory gland (AG), and Ance was shown to also be synthesised by this organ. Ance was found to be expressed in the secondary cells of the AG, specifically found enriched within the large vesicles of these cells (Rylett et al., 2007). ACE activity, determined by detecting HHL proteolysis, was lost from the AG during mating, consistent with the transfer of Ance to the mated female in the seminal fluid during copulation. Similar to mammalian ACE, Ance is thought to be important for the processing of biologically active peptides.

ACE2-deficient mice were reported to have marked defects in cardiac contractility (Crackower et al., 2002). A role for ACE2 in cardiac function is remarkably conserved in *Drosophila*. In fact, *Acer* mutants were found to have severe defects in heart morphogenesis during embryonic development (Crackower et al., 2002). Although the specificity of this phenotype was later disputed (Carhan et al., 2011), heart-specific knockdown of Acer expression was found to exacerbate the age-dependent decline in contractile parameters. Furthermore, reduced levels of Acer increased stress-induced heart failure rates and decreased fly lifespan (Liao et al., 2014). Other than a heart-related function, Acer also plays a role in regulating sleep behaviour in response to changes in nutrition (Glover et al., 2019). Mammalian ACE and ACE2 are expressed in adipose tissue, where a role in metabolic regulation has been suggested (Karlsson et al., 1998; Li et al., 2020). An overlap in the expression pattern is found in *Drosophila*, where Acer can be detected in the adult fat body of the head and abdomen (Carhan et al., 2011). Acer null flies develop normally but experience reduced night-time sleep and exhibit greater sleep fragmentation. Similar phenotypes were observed when flies were treated with Acer inhibitor fosinopril (Carhan et al., 2011). A follow-up study on these flies showed that different diets influenced sleep patterns (Glover et al., 2019). Loss of Acer was also found to disrupt nutrient-sensitive phenotypes including survival and glycogen storage. An alteration of the normal dietary regulation of the *Drosophila* insulin-like peptide 5 levels in *Acer* mutants suggests a role for Acer in the modulation of the insulin/IGF-like signalling pathway in the nutrient-dependent control of metabolism, sleep and survival (Glover et al., 2019).

In *Drosophila*, metamorphosis is accompanied by a three-fold increase in ACE activity compared to the levels observed in second and third instar larvae (Houard et al., 1998). Activity in newly eclosed adults then declines to that observed in pre-pupal stages. The observed increase in ACE activity, as measured by analysing HHL hydrolysis, is due to the induction of Ance expression in cells of imaginal discs in wandering third instar larvae. This induction is absent in mutants that fail to produce the ecdysone peak during the wandering larval phase, which is required to trigger puparium formation. The synthesis of ACE activity in addition to Ance protein expression brought about by physiological levels of 20-hydroxyecdysone in a wing disc cell line confirms that Ance is an ecdysteroid-responsive gene (Siviter et al., 2002b). It has thus been postulated that, during metamorphosis, Ance is required for the processing of a developmental peptide hormone or may function in concert with other peptidases to recycle amino acids from larval proteins for use in the synthesis of adult proteins (Siviter et al., 2002b). A recent study has dissected the mechanism through which expression of Ance is regulated in imaginal discs by revealing a requirement for Decapentaplegic (Dpp) signalling transcription factor Mad and GATA family transcription factor Pannier. This mechanism appears to be conserved in humans since ACE expression was found to be regulated by Mad and Pannier homologues SMAD2 and GATA4 (Kim et al., 2017).

ACE inhibitors and blockers are associated with a significant reduction in the incidence and progression of Alzheimer's disease (AD) (Davies et al., 2011; de Oliveira et al., 2014; Ho et al., 2017; O'Caoimh et al., 2014; Ohrui et al., 2004; Qiu et al., 2013; Soto et al., 2013; Wharton et al., 2015; Yasar et al., 2013). Interestingly, administration of ACE inhibitor captopril and angiotensin receptor blocker losartan was found to suppress neurodegenerative phenotypes in AD fly models, although neither drug affected the production, accumulation or clearance of Aβ42. Notably, neurodegenerative changes such as brain cell death and memory deficits were completely rescued in a homozygous *Acer* null background, hence demonstrating that the beneficial effect of captopril results from its targeting of Acer (Lee et al., 2020). In corroboration, an earlier study identified *Acer* and *Ance-5* as genetic modifiers of phenotypes

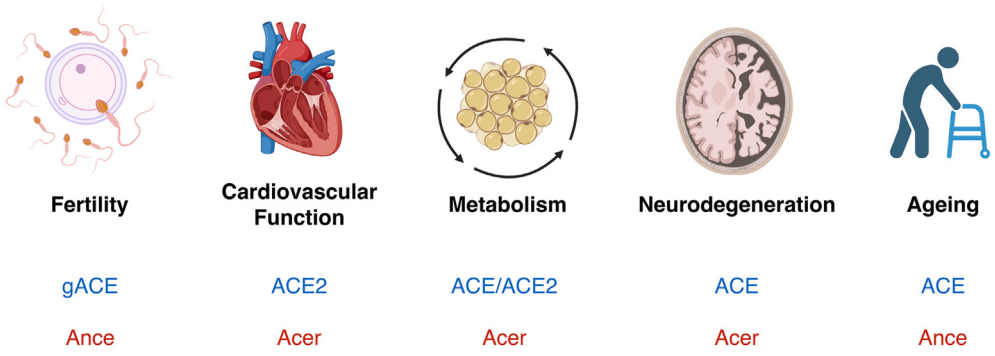

Figure 2. Shared functions of ACE proteins in humans and *Drosophila*. Functional studies in fruit flies have revealed five mechanisms in which there is an overlapping function between Ance or Acer and its human homologue. Functions, which might be conserved, are most probably RAS-independent considering that *Drosophila* has an open circulatory system. Figure created with BioRender.

associated with *Presenilin* and *Amyloid Precursor Protein* genes, mutations in which lead to early-onset autosomal dominant AD (van de Hoef et al., 2009). Considering that Ance is also a target of captopril (Kim et al., 2003), it remains to be seen whether Ance can also modify AD phenotypes in fly models. Treatment of flies with ACE inhibitor lisinopril was found to increase lifespan and physical performance, though the latter was genotype-dependent. Genotypes in which lisinopril was found to enhance motoric abilities had a reduction in age-related protein aggregation in muscle. Reduced levels of Ance in muscles achieved through RNAi-mediated knockdown abolished the effects of lisinopril on lifespan. This implies that the ameliorative effects of lisinopril on lifespan are most probably due to its targeting of Ance in muscle (Gabrawy et al., 2019). Lisinopril was also found to alter mitochondrial respiration and reactive oxygen species (ROS) levels in an age- and genotype-specific manner. These changes can also contribute to the drug's positive impact on age-related impairments (Ederer et al., 2018). All these findings indicate that modulation of RAS-independent functions can have a modifier effect on ageing and age-related diseases with implications for treatment development. Importantly, they highlight the need for continued exploration of the non-canonical functions of ACE and ACE2 with *Drosophila* showing great promise in this pursuit considering that this model organism has an open circulatory system (Figure 2).

5. Outstanding questions

Despite extensive research on ACE and ACE2 *Drosophila* orthologues Ance and Acer, several questions still remain to be answered (Box 1). The substrates that these two enzymes catalyse *in vivo* remain undiscovered. However, advances in proteomics aided by the availability of null mutants can help shed light on this gap in knowledge. Although *Drosophila* ACE-like proteins other than Ance and Acer are not predicted to be catalytically active, further research is warranted to test whether this hypothesis stands in an *in vivo* setting. Specifically, the functions of *Ance-2*, *Ance-3*, *Ance-4* and *Ance-5* in *Drosophila* remain unexplored and the availability of easy-to-use gene editing techniques including the CRISPR/Cas9 system to generate fly knockouts and/or RNAi transgenes, is expected to first and foremost determine whether these are essential genes. Secondly, such tools will allow us to identify tissue-specific roles. Few studies have thus far applied RNAi to select tissues. In one study, findings were crucial to confirm that the cardiac function ascribed for ACE2 is conserved for its Acer orthologue in *Drosophila* (Liao et al., 2014). Studies in the same vein will help us to not only define the unknown function of Ance-2 to Ance-5 but also to redefine the *in vivo* role of Ance and Acer. It would also be interesting to determine whether the two-domain membrane-anchored Ance-2+Ance-3 fusion protein is expressed in flies, potentially by generating and characterising flies with transgenic expression of this hypothetical protein thought to have the greatest structural resemblance to ACE and ACE2. Site-directed mutagenesis can also be employed to activate those ACE proteins thought to be catalytically inactive.

In addition to discovering possible genetic interactions, combinatorial mutant analysis will also help determine if mutant combinations are compatible with life, therefore, elucidating whether some ACE family members are redundant. This approach can also determine whether specific mutant-associated defects can be rescued in a combined mutant background similar to what was observed in *Ace/Ace2* double mutant mice (Crackower et al., 2002). Thus far, there is indication that double mutants for *Ance* and *Acer* are developmentally normal and fertile (Kim et al., 2017). Extensive genetic modifier screens can also reveal relevant pathways in mutants. The use of fluorescent tags, specific antibodies and immunofluorescence to identify tissue-specific expression patterns has already been quite informative in the study of Ance function (Carhan et al., 2011; Hurst et al., 2003; Kim et al., 2017; Rylett et al., 2007), and studies that apply these methods to the other ACE family members in *Drosophila* are expected to yield valuable results. This would be especially relevant for Ance-3 which is the only *Drosophila* ACE protein that is predicted to be membrane-bound in a similar manner to ACE and ACE2.

Given the societal impact of COVID-19, investigations that discover an overlap between the clinical features observed in COVID-19 patients, including those with long COVID, and phenotypes exhibited by flies with disruption of ACE family members will help elucidate the pathophysiology of this disease and its ramifications. It would also be interesting to determine whether ACE2 orthologues in *Drosophila*, like the murine ACE2 counterpart (Qiu et al., 2020; Wan et al., 2020), have a similar low binding affinity to the SARS-CoV-2 spike protein. Should this be the case, adopting the strategy utilised in mouse models (Jia et al., 2020; Munoz-Fontela et al., 2020), development of humanised ACE and ACE2 fly models through the transgenic expression of human ACE or ACE2 in either a wild-type or mutant background will be of great value to the research community. Such models will allow us to first determine whether the function of any or all ACE family members can be replaced by either or both human orthologues. Importantly, a transgenic ACE2 fly model can be a crucial tool to further understand ACE2 biology, investigate its role in the pathogenesis of coronavirus diseases including COVID-19 or its variations, and identify novel ACE2 inhibitors. The economical and expeditious platform afforded by *Drosophila* will be key for the discovery of treatments that complement those in the pipeline, making us more prepared for future coronavirus epidemics.

Box 1
Pending investigations in *Drosophila* aimed at better understanding the function of ACE proteins.

- Identification of substrates catalysed *in vivo* by Ance and Acer
- Characterisation of the unknown *in vivo* roles of Ance-2, Ance-3, Ance-4 and Ance-5
- Site-directed mutagenesis to activate ACE proteins thought to be catalytically inactive
- Detection and characterisation of two-domain membrane-anchored Ance-2 + Ance-3 fusion protein
- Determination of tissue-specific expression pattern of Acer, Ance-2, Ance-3, Ance-4 and Ance-5
- Establishing whether the absence of all ACE family members is compatible with life
- Application of genetic modifier screens to determine involvement in relevant pathways
- Binding affinity studies between ACE family members and SARS-CoV-2 spike protein
- Development of ACE and ACE2 humanised fly models

6. Conclusions

More than two decades have passed since the discovery of the first ACE family members in *Drosophila* and various studies have since shaped our understanding of their *in vivo* roles and the functional overlaps with human orthologues ACE and ACE2. The unprecedented scale of the COVID-19 pandemic, which has resulted in more than 200 million cases and 5.3 million deaths to date worldwide (Johns Hopkins University Coronavirus Resource Center: https://coronavirus.jhu.edu), has spurred us to re-evaluate the research done on ACE orthologues in *Drosophila*, aimed at determining whether the advantages offered by the fruit fly as a systems model can be exploited in our ongoing struggle against SARS-CoV-2 and its threatening vaccine-evading variants. We conclude that *Drosophila* provides an enticing opportunity to further our understanding of ACE as well as ACE2 in humans and the link of the latter to COVID-19. Identifying preventative and therapeutic interventions targeting ACE2 is feasible with the generation of humanised models being essential for this endeavour. We believe that research on ACE/ACE2 in flies provides an added value and, importantly allows us to be better prepared for the next coronavirus pandemic.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Data availability statement

No data was used for the research described in the article.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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