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Original article

Functional Analysis of GαO and InR in Regulating Longevity in Drosophila melanogaster

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Abstract

Aging is defined as a progressive loss of function, leading to reduced fertility, increased mortality, and disability [1]. Lifespan and healthspan are influenced by both genetic and environmental factors, and recent studies have shown that aging can be modulated in model organisms through genetic interventions [3]. In *C. elegans*, mutations in the *daf-2* insulin/IGF-1 receptor and *odr-3* G-protein-coupled receptor significantly extend lifespan, with the *daf-2; odr-3* double mutant showing synergistic effects beyond single mutants [2][3]. To expand this, we investigated the orthologous genes in *Drosophila melanogaster*: *InR* (insulin receptor) and *GαO* (G-protein alpha O subunit), using both single and double mutants. We found that both *InR* and *GαO* mutants extended lifespan compared to wildtype, however, the *GαO;InR* double mutant showed only a slight increase in lifespan compared to the *InR* mutant, less pronounced than the synergy observed in *C. elegans*. These findings suggest that while these genes may have conserved roles in lifespan regulation, their interactions differ between species, indicating species-specific modulation of aging.

Keywords

*Drosophila melanogaster***,** lifespan regulation, double mutants, GαO, Insulin/IGF-1 signaling.

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Introduction

Aging is a complex biological process governed by multiple genetic and environmental factors. In recent decades, model organisms such as *Drosophila melanogaster* and *Caenorhabditis elegans* have been instrumental in identifying longevity-associated genes (LAGs) and the molecular pathways they regulate. More than 2,200 single-gene interventions that modulate lifespan have been reported, many of which exhibit functional conservation across species [4]. However, while much is known about individual LAGs, the interaction effects between these genes, particularly in the context of lifespan extension, remain less well understood [10].

The insulin/IGF-1 signaling (IIS) pathway is one of the most well-characterized longevity pathways, with evidence from multiple species, including nematodes, flies, and mammals [4, 12]. In *Caenorhabditis elegans*, mutations in the IIS receptor *DAF-2* lead to a substantial extension in lifespan by activating the transcription factor *DAF-16* (a homolog of mammalian FOXO) [4]. Similarly, in *Drosophila melanogaster*, mutations in the insulin receptor gene *InR* (the *DAF-2* ortholog) significantly extend lifespan, with the underlying mechanisms involving a reduction in insulin-like signaling [9].

GαO is a G-protein alpha subunit involved in signal transduction via G-protein-coupled receptor (GPCR) pathways, playing a crucial role in neuronal signaling, locomotion, and sensory processes such as taste perception [7]. In *Drosophila*, it is predominantly expressed in the central nervous system and is essential for motor coordination, synaptogenesis, and neuronal differentiation [5]. Additionally, *GαO* mediates bitter taste sensitivity, linking it to key sensory pathways [7]. Although its direct role in lifespan regulation is not as well-established as *InR*, it is known that disruptions in *GαO* impact neurodevelopment and metabolic pathways, indicating its potential involvement in aging processes [5, 7].

This study focuses on understanding the genetic interaction between *InR* and *GαO* in *Drosophila melanogaster*, both individually and in combination. By assessing the lifespan of single and double mutants of these genes, we aim to investigate whether their combined effects on longevity are synergistic, dependent, or additive. Our findings contribute to the growing body of knowledge on the genetic regulation of aging and offer insights into the evolutionary conservation of lifespan determination mechanisms.

Materials and methods

Drosophila melanogaster strains and culture conditions

The *Drosophila melanogaster* strains utilized in this study were obtained from the Bloomington Drosophila Stock Center. These included the *GαO* mutant strain (y[1] w[67c23]; P{y[+mDint2] w[BR.E.BR]=SUPor-P}Galphao[KG01266]), the *InR* mutant strain (ry[506] P{ry[+t7.2]=PZ}InR[05545]/TM3, ry[RK] Sb[1] Ser[1]), and balancer/marker strains CyO, ROI (Df(2L)cl-h1/CyO, amos[Roi-1]), SM6 (dpy[ov1] Adc[b-1] cn[1] ptc[Conf-1]/SM6a), and TM3/TM6 (w[*]; TM3, Sb[1] Ser[1]/TM6B, Tb[1]). The Canton-S strain (Canton-S) was included as a control. Flies were reared in vials containing premixed Nutri-Fly™ Bloomington Formulation cornmeal medium composed of 84.18% water, 1.46% yeast, 0.84% soy flour, 6.15% cornmeal, 0.49% agar, 6.48% corn syrup, and 0.41% propionic acid.

Construction of lines of interest

To generate the double and triple mutants required for the lifespan assays, specific genetic crosses were performed. Approximately 30 virgin females and ~ 30 males were crossed and cultured together in bottles for 7 days at 25°C. After this period, the adult flies were removed. Upon hatching, the *Drosophila* progeny were validated through phenotypic markers and selected for subsequent crosses.

Crossing schemes for double and triple mutant construction

To generate the required double and triple mutants for the lifespan assays, a series of successive crosses were performed. The double mutants *GαO - InR*, *GαO - eIF*, and *eIF - InR*, as well as the triple mutant *GαO - eIF - InR*, were obtained through specific crossing schemes. Figure 1 outlines the main steps of the crosses for each genotype, as well as the overall experimental design. Straight lines and arrows indicate crosses between different lines, while self-loops represent mating within the same line.

Lifespan assay

Synchronized populations of *Drosophila melanogaster* (males and females in equal proportions) were collected and maintained at a population density of 27–32 individuals per vial. Flies were transferred to fresh vials containing new food every 2–3 days, and mortality was recorded throughout their adult lifespan. To avoid anesthesia-induced acute mortality, particularly in older flies, all transfers were performed without anesthesia. The age of the flies and the number of deaths were documented at each transfer.

Data analysis and statistics

Each group's median life span was calculated, and groups were compared using the log-rank test, plotting the survival curves based on recorded lifespan data, and the Gompertz-Makeham equation was used to reveal if an intervention alters lifespan by changing the rate of aging, or if it does so by altering health [6].

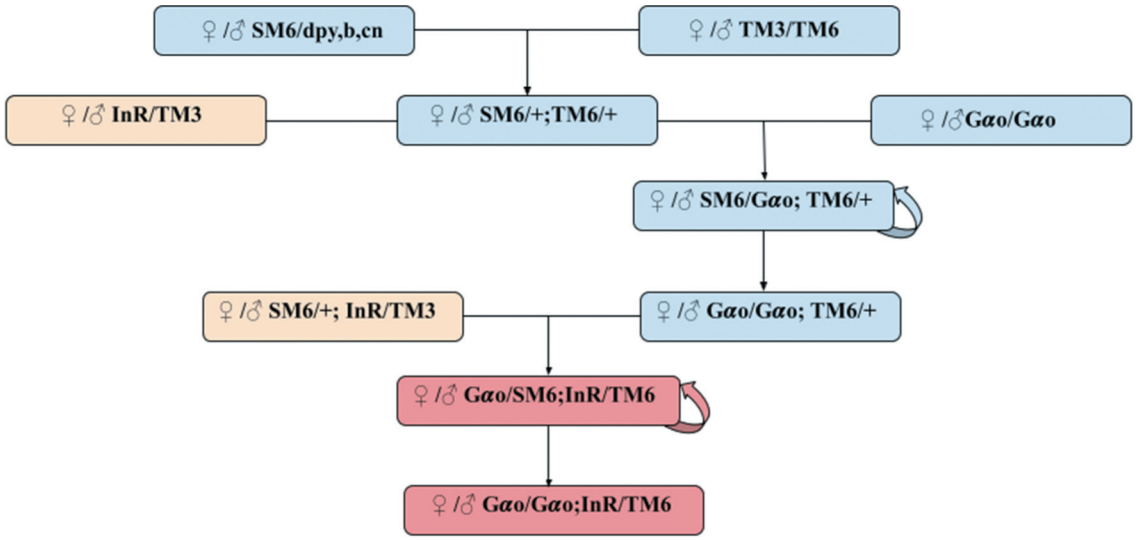

Figure 1. Crossing Scheme for the Generation of Double Mutant *GαO* - *InR*. Straight lines and arrows indicate crosses between different lines, while self-loops represent within-line mating.

Results

The lifespan of *Drosophila melanogaster* mutants *GαO*, *InR*, and their double mutant *GαO; InR* were compared to the wild-type control strain, Canton-S (Fig. 2). The maximum lifespan of the controls was 42 days (Fig. 2A-C). The *InR* mutant extended maximum lifespan moderately to 62 days (Fig. 2A; p-value <0.0001), while the *GαO* mutant showed a marked increase in maximum lifespan to 72 days (Fig. 2B; p-value <0.0001), indicating a significant extension. The double mutant *GαO;InR* exhibited a lifespan of 65 days, intermediate between the single mutants but shorter than *GαO* alone (Fig. 2C; p-value <0.0001).

Statistical analysis revealed a significant difference ($p < 0.0001$) between both the *InR* and *GαO* single mutants vs control, as well as between *InR* double mutant

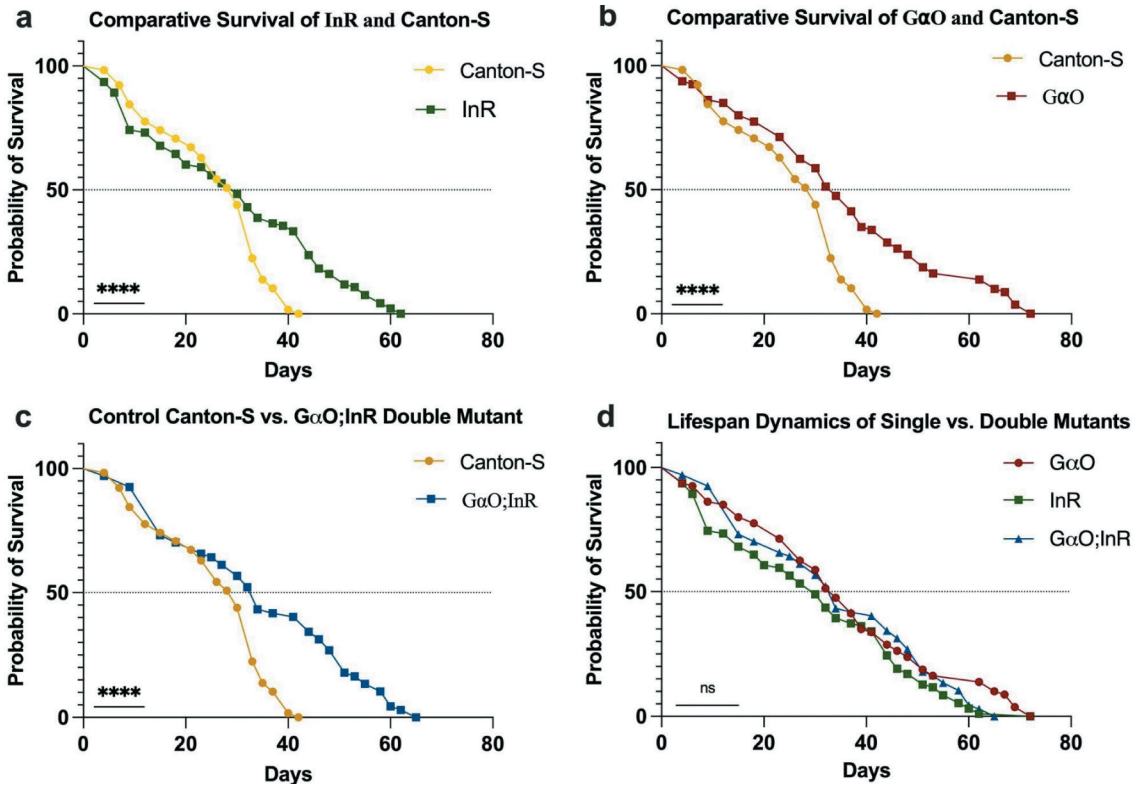

Figure 2. Survival Curves and Comparative Lifespan Analysis of Canton-S, *GαO*, *InR*, and *GαO;InR* Mutants: **(a)** Lifespan curves of the *Drosophila melanogaster* control strain Canton-S and InR mutant; **(b)** Comparative lifespan curves of Canton-S and *GαO* mutant; **(c)** Lifespan curves of Canton-S and *GαO;InR* double mutant. **(d)** Comparative survival of *InR* and *GαO* single mutants, and of the *InR* double mutant.

and control, suggesting that both interventions have a pro-longevity effect. However, a small statistically significant difference was observed between the two single mutants with InR mutants living slightly less ($p = 0.0145$), and no significant difference was observed between any single mutant and the double mutant ($p > 0.05$), indicating that the combination of these mutations does not further enhance lifespan beyond that conferred by *GαO* alone. This suggests that the effects of *GαO* and *InR* on lifespan are dependent, rather than synergistic [8].

Discussion

The distinct lifespan effects observed in this study for *GαO* and *InR* mutations in *Drosophila melanogaster*, suggest unique and potentially intersecting pathways through which these genes influence aging. Both mutants were generated by transposon insertion, which likely led to enhanced function in *GαO* and partial suppression of the insulin/IGF-1 signaling (IIS) pathway in *InR*. The *GαO* mutation significantly extended lifespan to 72 days, supporting the involvement of G-protein-coupled receptor (GPCR) signaling, a pathway integral to neuronal and metabolic regulation in aging [7, 9]. In contrast, the *InR* mutant extended lifespan more modestly to 62 days, consistent with its partial suppression of IIS, a pathway widely implicated in longevity regulation across multiple species [4, 12].

The lack of a synergistic effect in the double mutant (*GαO; InR*), which had a lifespan of 65 days, suggests that these genes might modulate lifespan through at least partly overlapping or dependent mechanisms. While *GαO* likely enhances neuronal signaling and metabolic processes that promote longevity, the partial suppression of IIS in the *InR* mutant does not appear to contribute further to lifespan extension when both genes are mutated. The non-significant differences between the single mutants and the *GαO;InR* double mutant, suggest a dependent effect [8]. This indicates that the lifespan extension conferred by these mutations reaches a threshold, with no further benefits achieved when both genes are altered simultaneously. This type of interaction is supported by insights from the SynergyAge database, which highlights the large fraction of non-additive genetic interactions as a general characteristic across aging-related pathways [8].

The interplay between IIS and GPCR pathways, both of which are involved in metabolism, stress responses, and neuronal function, could explain the absence of additive effects in the double mutant. These pathways are likely converging on similar targets related to aging regulation, limiting the lifespan benefits when both are mutated. This hypothesis is further supported by insights from the SynergyAge database, which indicates non-additive interactions between these genes in lifespan regulation [8, 10]. Our results contribute to the understanding of genetic interactions in longevity, highlighting the need for further investigation into how *GαO* and *InR* interact at a molecular level. Future studies should also consider the role of external factors, such as the gut microbiome, in modulating these genetic effects on lifespan [9].

Conclusion

This study is the first to evaluate the role of *GαO* in lifespan regulation alongside *InR* in *Drosophila melanogaster*. Both mutations independently extended lifespan, with *GαO* showing a significant increase, likely due to enhanced GPCR signaling, and *InR* contributing moderately by partially suppressing the IIS pathway. However, their combination in the double mutant did not yield further lifespan extension compared to single mutants, suggesting the two genes/pathways function through partly overlapping or dependent pathways. While our findings provide important insights into these interactions, the use of transposon insertions which was used for gene modulation limits precision in gene function alteration. Future studies could benefit from using the GAL4/UAS system or CRISPR-Cas9 to achieve more targeted overexpression or downregulation of *GαO* and *InR*, which in turn would better clarify the individual contribution of each gene to aging.

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Conflicts of interest

The authors declare no conflict of interest.

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