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GHRELIN: A NOVEL THERAPEUTIC STRATEGY AGAINST THE PREMATURE AND ACCELERATED AGING CLOCK PHENOTYPE IN PROGERIA EXPERIMENTAL MODELS

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INTRODUCTION

WHAT IS PROGERIA?

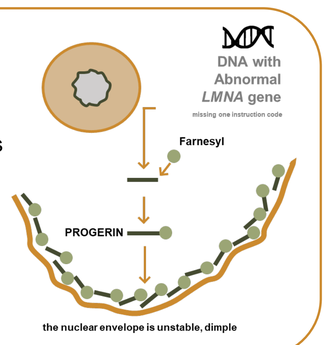
Progeria, also known as Hutchinson-Gilford Progeria Syndrome (HGPS), is a rare, fatal genetic condition of accelerated aging in children.

Without treatment, children with Progeria die of heart disease at na average age of 14.5 years.

1 in 18 million people have Progeria. As of April 2022, PRF knows of more than 144 cases in 50 countries.

WHAT CAUSES PROGERIA?

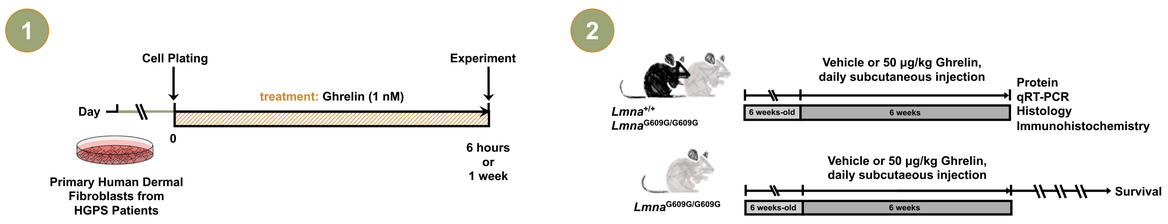
A mutation in the *LMNA* gene creates a protein, called **progerin**, which makes the nucleus of a cell unstable and causes premature aging.



- Cardiovascular dysfunction
- Metabolic dysfunction
- Skin abnormalities
- Musculoskeletal dysfunction
- LIPODYSTRHOPHY

AIM: To investigate the therapeutic potential of ghrelin in delaying Hutchinson-Gilford progeria syndrome (HGPS) aging phenotype by elucidating its effects on i) cellular aging mechanisms; ii) the whole-body aging phenotype; and iii) lifespan in a HGPS mouse model (*Lmna*$^{G609G/G609G}$ mice).

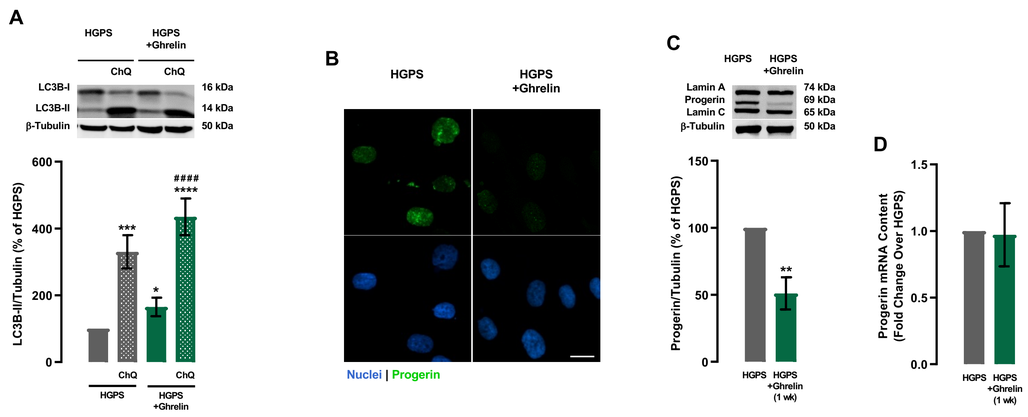
METHODOLOGIES



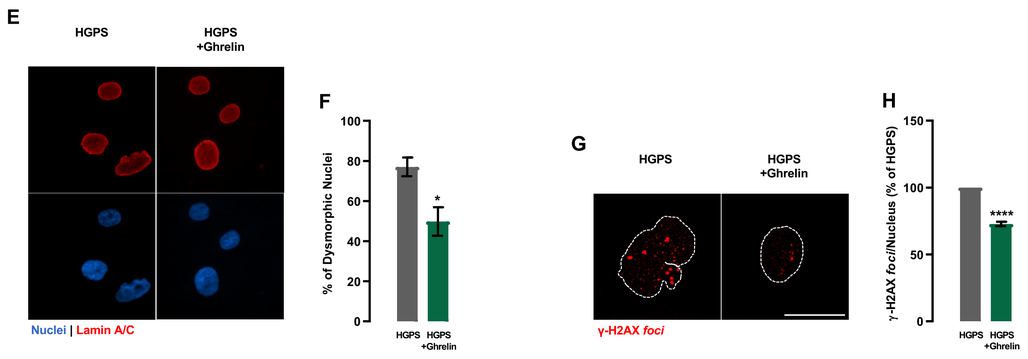
RESULTS

1

Ghrelin enhances autophagy and progerin clearance in HGPS fibroblasts



Ghrelin rescues nuclear morphology and decreases DNA damage in HGPS fibroblasts



Ghrelin enhances cell proliferation and delays cellular senescence in HGPS fibroblasts

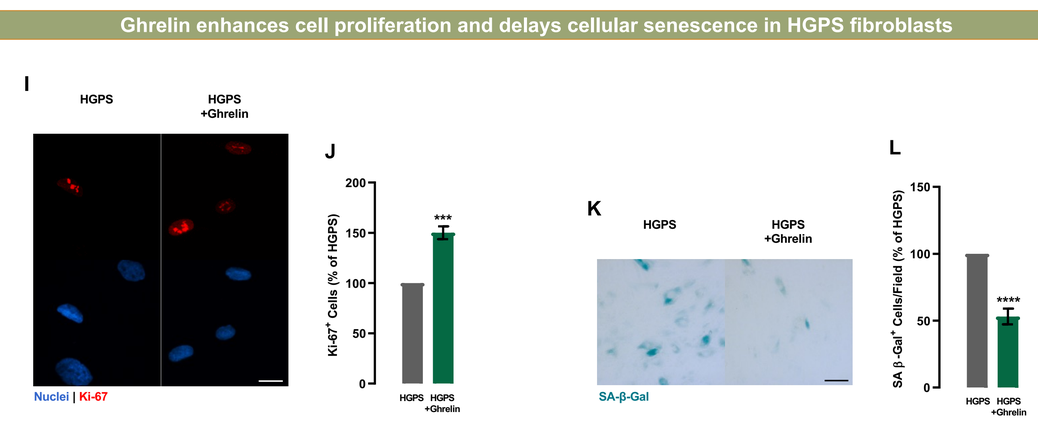
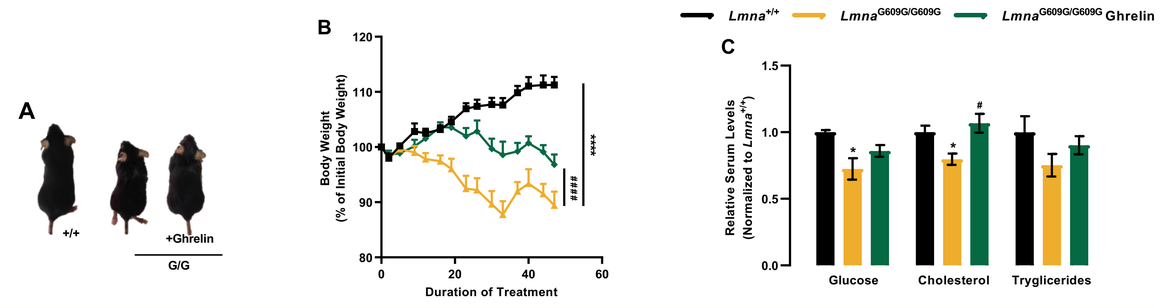


FIGURE 1 (A-D) Ghrelin enhances autophagy and progerin clearance in HGPS fibroblasts. Whole-cell extracts were assayed for LC3B **(A)** (*N*=4), Lamin A/Progerin/Lamin C **(C)** (*N*=5) and β-Tubulin (loading control) immunoreactivity through Western blotting analysis. **(B)** Ghrelin decreased progerin immunoreactivity. Cells were immunolabeled for progerin (top panels, green) and nuclei were stained with Hoechst 33342 (bottom panels, blue). Scale bar, 10 µm. **(D)** Quantitative polymerase chain reaction analysis of progerin mRNA levels in HGPS fibroblasts upon 1 week of ghrelin treatment. **(E-H) Ghrelin delays cellular senescence in HGPS fibroblasts**. **(E)** HGPS fibroblasts were immunolabeled for Lamin A/C (red, top panel) and nuclei were stained with Hoechst 33342 (blue, bottom panel) (A). Scale bar, 10 µm. Quantification of the number of misshapen nuclei **(F)** upon ghrelin treatment (*N*=4-6). **(G and H)** Ghrelin decreased γ-H2AX immunoreactivity. Cells were immunolabeled for γ-H2AX (red), scale bar, 20 µm **(G)**. Quantification of γ-H2AX *foci* number **(H)**. **(I-L) Ghrelin increases cell proliferation and delays cellular senescence. (I and J)** Cells were immunolabeled for Ki-67 (red, top panel) and nuclei were stained with Hoechst (blue, bottom panel), scale bar, 10 µm **(I)**. Quantification of the number of Ki-67-positive cells in HGPS and ghrelin-treated HGPS cells **(J)**. **(K and L)** Ghrelin decreases cellular senescence, as determined by SA-β-Gal activity, scale bar, 100 µm **(K)**. Quantification of SA-β-Gal-positive cells **(L)**. Data are expressed as the mean±SEM of at least three independent experiments and are expressed as a percentage of HGPS. *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001 significantly different compared to HGPS; ####P<0.0001, significantly different compared to HGPS+Ghrelin, as determined by analysis of variance, followed by Tukey's multiple comparison test, or Student's *t* test. HGPS = Hutchinson-Gilford progeria syndrome.

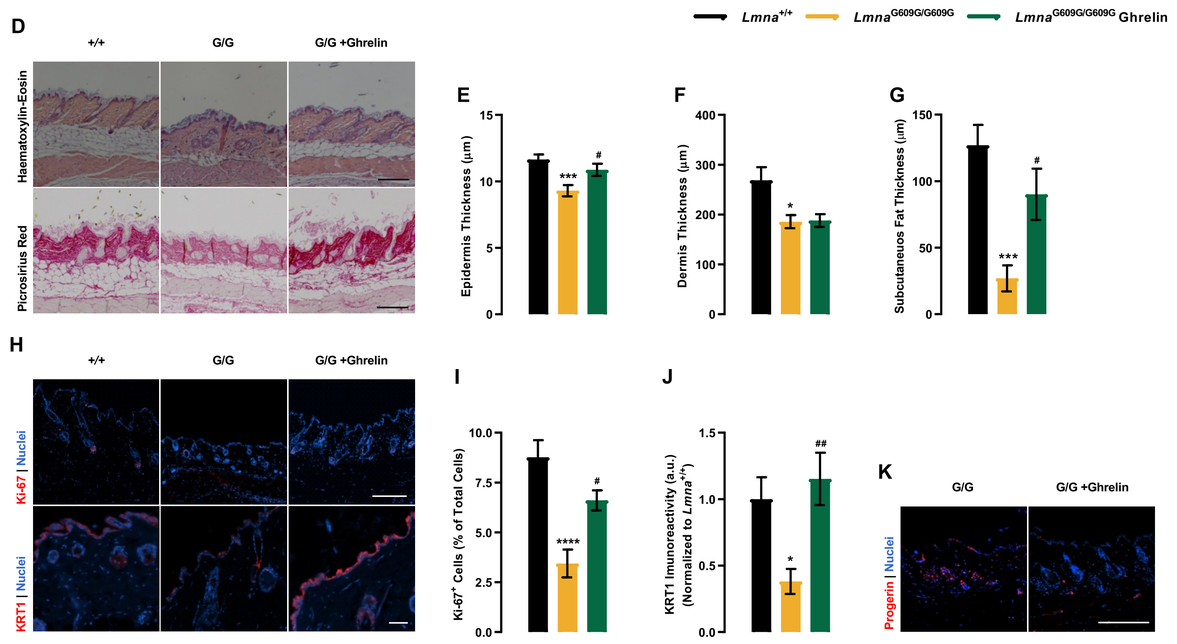
RESULTS

2

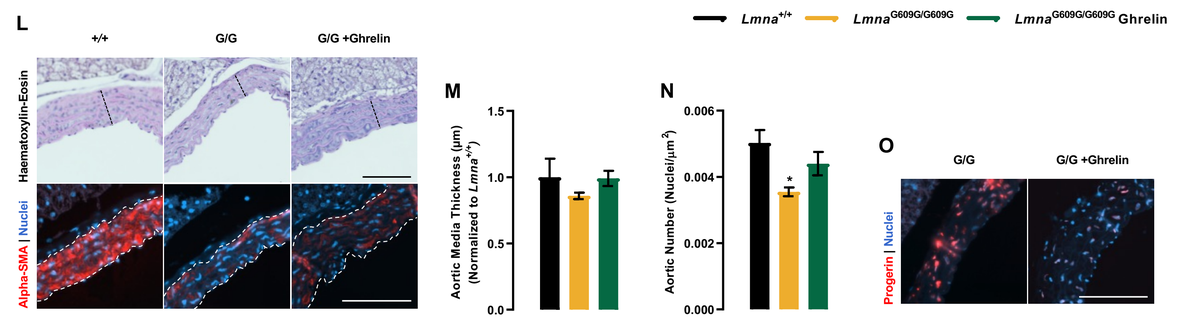
Ghrelin treatment directly affects *Lmna*$^{G609G/G609G}$ phenotype



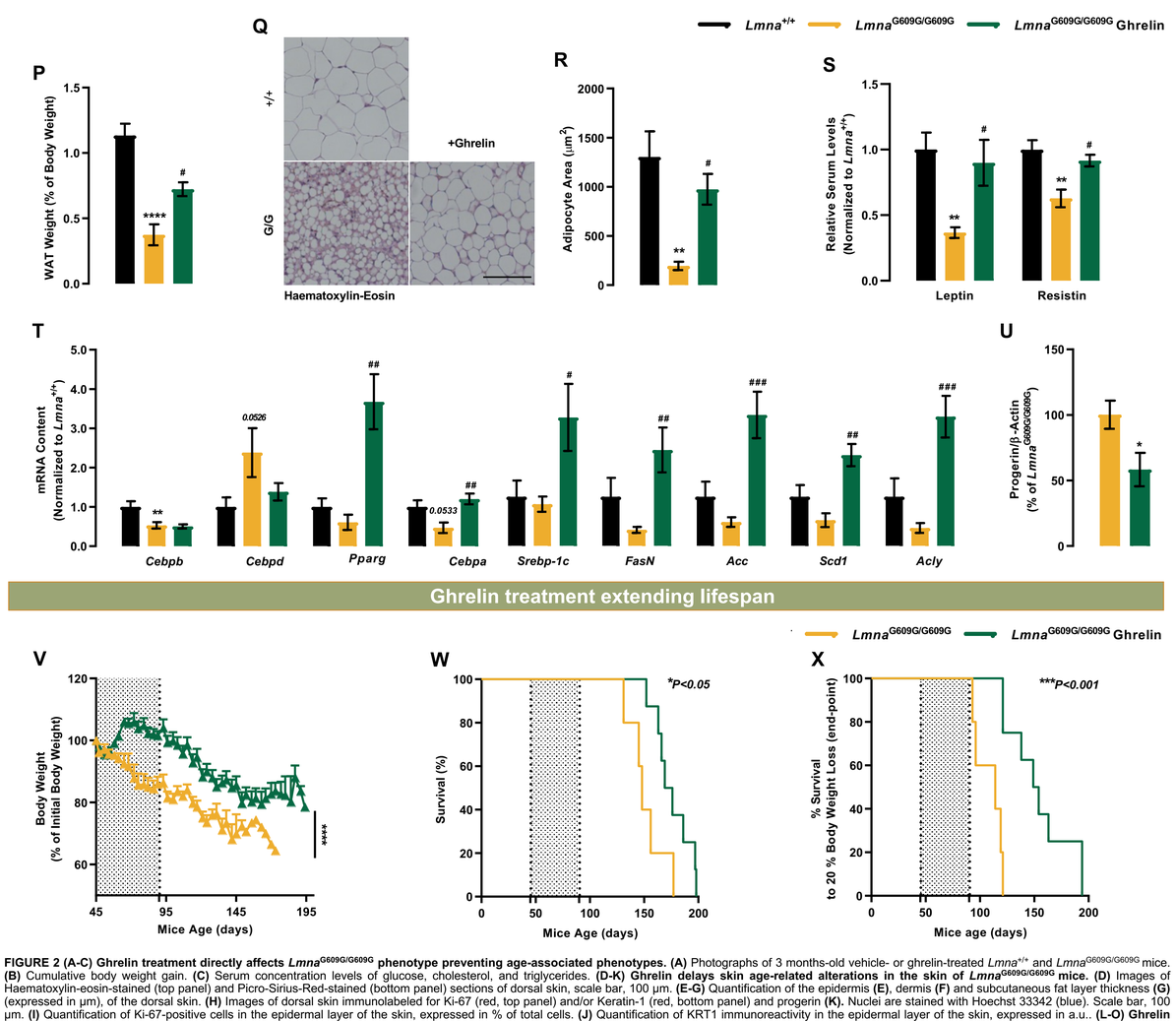
Ghrelin delays skin age-related alterations in the skin



Ghrelin ameliorates cardiac related-pathology



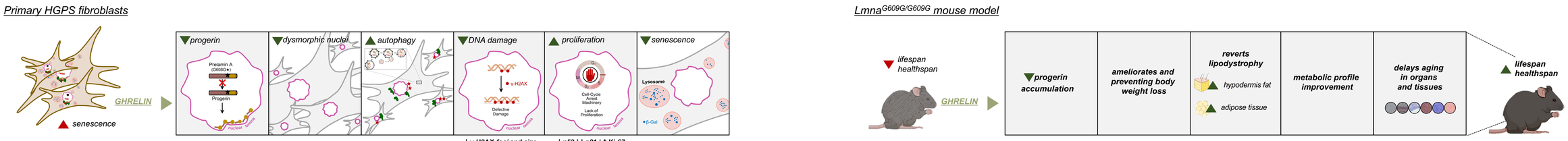
Ghrelin alleviates pathological changes in fat distribution and reverts lipodystrophy



Ghrelin treatment extending lifespan

FIGURE 2 (A-C) Ghrelin treatment directly affects *Lmna*$^{G609G/G609G}$ phenotype preventing age-associated phenotypes. (A) Photographs of 3 months-old vehicle- or ghrelin-treated *Lmna*$^{+/+}$ and *Lmna*$^{G609G/G609G}$ mice. **(B)** Cumulative body weight gain. **(C)** Serum concentration levels of glucose, cholesterol, and triglycerides. **(D-K) Ghrelin delays skin age-related alterations in the skin of *Lmna*$^{G609G/G609G}$ mice. (D)** Images of Haematoxylin-eosin-stained (top panel) and Picro-Sirius-Red-stained (bottom panel) sections of dorsal skin, scale bar, 100 µm. **(E-G)** Quantification of the epidermis **(E)**, dermis **(F)** and subcutaneous fat layer thickness **(G)** (expressed in µm), of the dorsal skin. **(H)** Images of dorsal skin immunolabeled for Ki-67 (red, top panel) and/or Keratin-1 (red, bottom panel) and progerin **(K).** Nuclei are stained with Hoechst 33342 (blue). Scale bar, 100 µm. **(I)** Quantification of Ki-67-positive cells in the epidermal layer of the skin, expressed in % of total cells. **(J)** Quantification of KRT1 immunoreactivity in the epidermal layer of the skin, expressed in a.u.. **(L-O) Ghrelin ameliorates cardiac related-pathology of *Lmna*$^{G609G/G609G}$ mice.** Images of Haematoxylin-eosin- **(L**, top panel) alfa-smooth muscle actin, α-SMA, (**L**, red, bottom panel), and progerin (**O**, red) stained cross-sections of the aorta. Nuclei are stained with Hoechst 33342 (blue). Scale bar, 100 µm. Quantification of aortic media wall thickness, expressed in µm **(M)**, and aortic wall nuclei number, expressed as aortic wall nuclei number/mm^2 **(N)**. **(P-U) Ghrelin alleviates pathological changes in fat distribution and reverts the progerin-impacted transcription of core adipogenic regulators during adipocyte differentiation of *Lmna*$^{G609G/G609G}$. (P)** Size of the gonadal white adipose tissue (WAT), expressed as a percentage of % of body weight. **(Q)** Images of Haematoxylin-Eosin-stained sections of WAT, scale bar, 100 µm. **(R)** Quantification of adipocyte area (µm^2). **(S)** Serum concentration levels of leptin and resistin. **(T)** Quantitative polymerase chain reaction analysis mRNA levels of WAT adipogenic differentiation genes (*Cebpb*, *Cebpd* (early differentiation regulators), *Ppary* and *Cebpab* (late differentiation regulators)), lipogenic genes (*Srebp-1c*, *FasN, Acc, Scd1* and *Acly*), fatty acid β-oxidation (*Mcad* and *Cpt1*) and gluconeogenic gene (*Pepck*). **(U)** WAT protein lysates were assayed for Lamin A/Progerin/Lamin C and β-Actin (loading control) immunoreactivity through Western blotting analysis. Data are expressed as the mean±SEM. *N*=4-19 *per* group. *P<0.05 ***P<0.001 and ****P<0.0001, significantly different from *Lmna*$^{+/+}$ mice or *Lmna*$^{G609G/G609G}$ mice; #P<0.05, ###P<0.01 and ####P<0.001, significantly different compared to *Lmna*$^{G609G/G609G}$ mice, as determined by one analysis of variance, followed Tukey's multiple comparison test or Student's *t* test. **(V-X) Ghrelin treatment extending lifespan. (V)** Cumulative body weight gain, as the percentage of weight gain between the beginning and the end of the study. **(W)** Kaplan-Meier survival plots. **(X)** Kaplan-Meier survival plots based on 20 % body weight loss showing a 37 % increase in Kaplan-Meier area under the curve. Data are expressed as the mean±SEM. *N*=5-8 *per* group. *P<0.05 and ***P<0.001, significantly different compared to *Lmna*$^{G609G/G609G}$ mice, as determined by Student's *t* test and Log-rank/Mantel-Cox test; Chi-Square 5.19 and 11.11 for graphs **(W)** and **(X)**, respectively.

CONCLUSIONS



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