IGF-1 and aging
Do we age at different rates?
Aging is the major risk factor for death from **ALL** chronic diseases
Low GH/IGF-1 levels/action models
Genetics of human longevity
Can a study design depict the challenges of genetics of aging?

- Only ~1/10,000 individuals is 100 years old (>520; 95-112)

- There is a remarkable family history of exceptional longevity in parents, siblings and offspring of “centenarians”

- Are there inherited protective factors that can assure human’s longevity?
A major barrier to conducting a study of centenarians

Is there an appropriate control group?

• The cohort assembled to date ("LonGenity") is unique:

• Offspring of centenarians are enriched with longevity phenotypes and genotypes

• Study of offspring permits comparisons with age- and gender-matched controls.

• All Ashkenazi Jews
90 years before

Cover of PLoS Biology April 2006
Modeling changes in the frequency of a genotype as a function of age

- Longevity genotypes
- Aging or “killing” genotypes
- Genes not associated with life-span
Frequency trends of favorable longevity Genotypes/Allele (All validated or have phenotype/function)
Partial list

**Longevity Gene Project (98)**
- Gil Atzmon Ph.D.
- Richard Lipton MD
- Clyde Schechter MD
- Magda Gabriely MD
- Marielisa Rincon MD
- Bill Greiner R.N.
- Debra Davidson M.Sc.
- Jeff Bauman MD
- Glenn Siegal MD
- Ms. Marion Maze
- Ms. Linda Radner
- Ms. Orit Ben David
- Swapnil Rajpathak MD
- Ilan Gabriely MD

**LonGenity (P01-07)**
- Projects:
  - P#1 Aviv Bergman Ph.D
  - P#2 Yousin Suh Ph.D
  - Hassy Cohen MD (UCLA)
  - P#3 Swapnil Rajpathak MD
  - Jill Crandall MD
  - Judy-W-Rossett PhD
  - P#4 Richard Lipton M.D

**Cores:**
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- M Katz,, J Schein ML Giraldi,
- D Green, W Guzman,
- G Trandafirescu, S Alcala
- A Russo, D Sparacio
- V Tabatabae

**Statistical:** Mimi Kim Ph.D
- Kenny Ye PhD
- Charley Hall PhD
- Xin Zheng

**Genetic:** Gil Atzmon PhD

**The core of laboratory (R01/+)**
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- Colette Knight MD
- Derek Huffman PhD
- Francine Einstein MD
- Reid Thompson MSTP
- Temuri Budagov M.S
- HongXiang Li
- Ling Guan Cui

**Other Collaborators**
- Amir Lerman (Mayo Clinic)
- Sree Nair (Mayo Clinic)
- John Greally MD PhD (Rinstein)
- Alan Shuldiner M.D. (UMD)
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- Ben Glazer MD (Hadassah)
- Zohar Nir PhD (Negev)
- Josephe Attardi Ph.D. (Cal Tech)
- Tom Pearls (BU)
- Eline Slogbloom PhD (Leidin)
- Rudy Westerndorp MD (Leidin)

**Support: NIH**: R01, P01 (genes), P01 (metabolic), K08.
Others: Glenn Foundation AFAR, Ellison Medical Foundation, LWPES, DRTC,
IGF-1 levels in centenarians, their children, and age-gender-matched control (UCLA ELISA)

Male, \( r = -0.52 \), \( p < 0.0001 \)

Female, \( r = -0.28 \), \( p < 0.0001 \)
Female Offspring have higher IGF-1

Female Offspring are shorter

IGF-1 (ng/ml)

Longevity in IGF-1R het-KOs

(Holzenberger, Nature 2003)

Mice are slightly smaller and have higher IGF-1 levels
Males do not live longer
IGF1R domain structure and coding variants

- SNPs in centenarians
- SNPs related to IR and growth retardation

Yousin Suh PhD
Jing Huang, Ph.D

Genotyping IGF1R Mutations in the full groups (n=700) revealed 7 centenarians vs. 1 control (~2%) harbor nonsynonymous mutations (p<0.02) and carriers have higher IGF-I ((p<0.04) and tend to be shorter

Reduced receptor number

Reduced IGF1R signalling

IGF1R (AU)

IGF-1-induced Phos/total AKT ratio

Impaired IGF-1R signaling in cells expressing longevity associated human IGF-1R alleles.
(Tazearslan, Huang, Barzilai and Suh. Aging Cell in press)
The GH receptor

D3GHR homozygosity is present in 6-12% of Europeans.

The exon 3 deletion of the GHR was linked to the growth response in ISS (Dos Santos; Nature Genetics 2004)

Additional studies are not uniformly confirmatory

(Science 255, 1992)
Percentage of GHRd3 homozygote isoform in males centenarians and their offspring and unrelated controls.

*p < 0.05 vs. control*
Prevalence of d3GHR HOMOZYGOSITY with aging

Slope:
\[ \beta = 0.21, \ p = 0.017 \]
\[ r = 0.58, \ p = 0.017 \]

* p<0.05 vs. 65
Age trend of the d3GHR isoform in 2 independent populations
Atzmon et al (in preparation)
Phenotypic associations with D3GHR homozygote isoform in Centenarian

**Lower IGF-1 levels**

<table>
<thead>
<tr>
<th>Serum IGF-1 (ng/dL)</th>
<th>D3GHR carrier</th>
<th>D3GHR non-carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
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<tr>
<td>100</td>
<td></td>
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<tr>
<td>140</td>
<td></td>
<td></td>
</tr>
<tr>
<td>180</td>
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</table>

*p<0.003

**Height (inches)**

<table>
<thead>
<tr>
<th>Height (inches)</th>
<th>D3GHR carrier</th>
<th>D3GHR non-carrier</th>
</tr>
</thead>
<tbody>
<tr>
<td>63</td>
<td>*</td>
<td></td>
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<tr>
<td>65</td>
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<td>67</td>
<td></td>
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<tr>
<td>69</td>
<td></td>
<td></td>
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<tr>
<td>71</td>
<td></td>
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</tbody>
</table>

*p<0.03

Thus it appears that in the elderly, the d3GHR polymorphism is associated with reduced GH action, possibly through reduced GH secretion as a result of a feedback mechanism.
Growth hormone receptor deficiency (Laron Dwarfism) is associated with a major reduction in pro-aging signaling, cancer, and diabetes in humans. Sci Transl Med. 2011 Feb V. Longo’s group in USC

While the frequency of diseases such as cancer is different in this population, their life expectancy is similar to the general population
How can we identify longevity genes with an un-biased approach?

- High throughput technology
- Statistical methods
- Many subjects

Gil Atzmon, kenny Ye, and Aviv Bergman
Manhattan plot for longevity GWAS (Affi 6.0) of ~100 yo vs. ~70 yu (N=350 in each group)
GWAS for Longevity (SNPS with $P < 10^{-6}$) (centenarians vs. unrelated 70yo)

- Histone deacetylase 8
- Sarcoglycan zeta
- Ribosomal protein S26
- TAF7-like RNA polymerase IIv
- Protein kinase, cGMP-dependent
- POU domain, C-3 transcription factor 1
- Heparan sulphate
IGF-I and risk of age-associated diseases
prospective population-based case-control studies

Chan et al, 2002
Harman et al, 2000
Stattin et al, 2000
Stattin et al, 2000 (<59yrs)
Hankinson et al, 1998
Hankinson et al, 1998 (<50yrs)
Toniolo et al, 2000
Toniolo et al, 2000 (premenopausal)
Zhao et al, 2003
Giovannucci et al, 2000
Kaaks et al, 2001
Ma et al, 1999
Probst-Hensch et al, 2001
Lukanova et al, 2000
London et al, 2002

Prostate cancer
Breast cancer
Bladder cancer
Colorectal cancer
Lung cancer

Maybe a genotype protective against T2DM CVD etc… is needed

Garnero et al, 2000
Sandhu et al, 2002
Juul et al, 2002

Adjusted Relative Risk

0.1 1 10
Regulation of Glucose Homeostasis

Glucose production

Glucose uptake

Lipolysis

Insulin Secretion

Energy balance
Body fat distribution
(leptin
Beta3 Adrenoreceptor)

IGF-1 and body composition?
(Why its important?)
Experimental Protocol

Stereotactic placement of infusion cannulae into the MBH

![Diagram of stereotactic placement](image)
Effect of ICV IGF-1 on HGP

* P<0.02

Same as peripheral

Muzumdar et al Diabetes 2006 ;55(10):2788-96
Central hIGF-1 (ICV 7-10d) decreases visceral fat

Perinephric fat (g)

- Young
- Old

- aCSF
- IGF-1

*p < 0.05
Strategy to prevent age-related diseases with IGF-1

Increase IGF-1 to brain

Decrease IGF-1 to periphery

Neural protection (AD)
Insulin sensitization (DM)
Body composition
Protection: IHD, osteop ?

Mainly central (?)

Peripheral

Cancer

Longevity

Longevity
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Internal Advisory Board:
Smoller, Shamoon, Fleischer

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LWPES, DRTC,
I want to go to Ventura!

In post developed animals (not genetic/intrauterine)
Observation: IV, physiological infusion of IGF-1 Suppresses Hepatic Glucose Production

• Where does IGF-1 act?
• There are scarce receptors for IGF-1 in the hepatocytes.

• Is it through CNS?
• Leptin, nutrients and insulin affect glucose metabolism through the hypothalamus.
ICV surgery

IV catheter

Day -14

Day -3

Day 0

Day of the clamp

ICV (aCSF/scrambled peptide) and/or IV infusions

25% dextrose

Somatostatin

Insulin 3mu/kg/min

Tritiated glucose infusion
Genotyping the two isoform of the GHR gene

Pantel et.al. JBC, 2000.
Effects of GHR polymorphism on GH responsiveness
Dos Santos et al. Nature Genetics 2004

Confirmed the D3GHR to be present in 30% of Europeans. The exon 3 deletion of the GHR was linked to the growth response in ISS.
What else do we know of D3GHR?

• No effect on growth in SGA (Carrascosa et al. JCEM 2006)
• No effect on growth in ISS (Hujeirat et al. HR 2006)
• No effect on growth in GHD (Pilota et al. JCEM 2006)
• No effect on growth in iGHD (Blum et al. JCEM 2006)
• contributes to growth in TS. (Binder et al., JCEM. 2006)
• contributes to growth in SGA. (Audi et al., JCEM. 2006)
• Associates with pre + post natal growth (Jensen et al., JCEM. 2007 )
• Higher response/taller final Ht in GH Rx of GHD (Jorge, JCEM 2006 )
• Reduced risk of T2DM (Strawbridge et al., GHIGFR. 2007)
• Protective effect on breast cancer risk (Wagner, IJC. 2006)
Prevalence of GHRd3 in various ages

Slope:
\[
\beta = 0.21, \ p = 0.017
\]
\[
r = 0.58, \ p = 0.017
\]

* \(p < 0.05\) vs. 65
hGHBP (pM) Adj. for age and gender in carriers and non-carriers of the d3-GHR

* p=0.008 vs. non-carrier
In vitro effect of GH stimulation on proliferation of transformed lymphocytes from male centenarians

In the graph:
- The x-axis represents GH concentrations (100 ng/ml).
- The y-axis represents Growth AU.
- Black bars represent fl/fl n=10, and red bars represent d3/d3 n=10.
- A statistical significance is indicated by asterisks: * for p<0.05, ** for p<0.01.
Phosphorylation of ERK in normal fl/fl and d3-GHR transformed lymphocytes from male centenarians.

Similar to a recent description of a GHSR. Pantel JCI
No effect on the phosphorylation of Akt and Stat 5 in transformed lymphocytes from male centenarians

This demonstrates a unique, pathway-specific effect of the d3GHR polymorphism on GH signaling with decreased constitutive activation but enhanced GH-driven signaling.
Centenarians interaction with the environment  
(n=477, 75% females)

<table>
<thead>
<tr>
<th>'Environmental' risk</th>
<th>Centenarians</th>
<th>NHANES1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Men</td>
<td>Women</td>
</tr>
<tr>
<td>Over weight</td>
<td>43%</td>
<td>34%</td>
</tr>
<tr>
<td>Smoking</td>
<td>60%</td>
<td>30%</td>
</tr>
<tr>
<td>Alcohol (daily)</td>
<td>24%</td>
<td>12%</td>
</tr>
<tr>
<td>NOT Exercise</td>
<td>55%</td>
<td>79%</td>
</tr>
</tbody>
</table>

(strenuous: tennis, baseball, swimming)

Vegetarians: 2.6%

Jill Crandall and Swapnil Rajpathak