The IRS2 Gly1057Asp Variant Is Associated With Human Longevity

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Background. Reduced insulin and insulin-like growth factor-1 (IGF-1) signaling extends the life span of invertebrate and mammals. Recently, reduced insulin receptor substrate-2 (IRS2) signaling was found associated with increased longevity in mice. The aim of our study was to evaluate whether a common polymorphism (Gly1057Asp) in human IRS2 gene is associated with human longevity.

Methods. Six hundred seventy-seven participants (289 males and 388 females) between 16 and 104 years of age, categorized as long lived (LL; >85 years old) or controls (C; <85 years old), were genotyped for Gly1057Asp-IRS2 locus variability (rs1805097). All participants, contacted at home or in their institution or selected from Italian geriatric and internal medicine or geriatric rehabilitation structures, underwent to a clinical, biochemical, and functional characterization, with particular attention to the insulin and IGF-1 signaling. Insulin resistance (Homeostasis Model Assessment [HOMA]-IR), insulin sensitivity (HOMA IS), and ß-cell function (HOMA-B cell) were calculated by the HOMA2 calculator v2.2 (www.dtu.ox.ac.uk/homa).

Results. In the whole population, homozygous IRS2gly/Asp participants were more represented among LL versus C participants (16.7% vs 12.0%; p = .04). The association between IRS2 gene polymorphism with longevity (being LL) was independent of anthropometric and metabolic covariates (odds ratio: 2.07, 95% confidence interval [CI] = 1.38–3.12; p = .001). Categorizing participants into percentiles by age, IRS2gly/Asp participants were more likely to reach extreme old age (≥90 percentile, 96–104 years; odds ratio: 2.03, 95% CI = 1.39–2.99; p = .0003).

Conclusions. These results support the hypothesis that the IRS2 branch of the insulin and IGF signaling is associated with human longevity. Further studies will be necessary for replicating our finding in an independent larger population group with sufficient power before the association between IRS2 gene polymorphism and longevity can be regarded as proven. Furthermore, studies of genetic and/or environmental background interactions may be useful after basic replication is complete.

Key Words: IRS2 gene polymorphism—Longevity—Insulin signaling.

The potential link between aging and insulin and insulin-like growth factor-1 (IGF-1) signaling attracted substantial attention on the basis of several evidences showing that disruption of insulin and IGF-1 signaling cascade can significantly extend life span in diverse living species from yeast to rodents (1–4). A recent study demonstrated that systemic or neural-specific reduction of the insulin receptor substrate-2 (IRS2) can extend the life span of mice (5). However, it is difficult to determine directly whether IRS2 signaling regulates human longevity. In humans, a number of polymorphisms have been identified in the IRS2 gene, including Gly1057Asp variant that occurs with an allelic frequency of 34% (6). Some, but not all, studies have indicated a role for such variant in the pathogenesis of obesity and obesity-associated insulin resistance (6–12). Furthermore, in some but not all populations, obesity and IRS2gly/Asp have been found associated with infertility, reduced pancreatic ß-cell function, and increased type 2 diabetes susceptibility (6–12).

Interestingly, IRS2 knockout mice display similar characteristics, including infertility, reduced ß-cell mass and insulin secretion, and an increased peripheral insulin resistance that predisposes to diabetes (5). By contrast, a hemizygous IRS2 deletion causes mild glucose intolerance until middle age. Conversely, old hemizygous mice are more insulin sensitive and glucose tolerant than wild-type mice (5). Moreover, IRS2+/− mice live nearly 18% longer than wild-type mice (5).

In humans, insulin resistance ordinarily develops with age and is an important risk factor for various metabolic disorders—hypertension, atherosclerosis, obesity, and type 2 diabetes—strongly affecting morbidity, disability, and mortality among the elderly participant (13). By contrast, people aged between 85 and 90 years and older display relatively high insulin sensitivity and glucose tolerance, and centenarians are remarkably insulin sensitive (14). Because the metabolic profile of long-lived participants resembles that of IRS2+/− mice, we investigated whether the IRS2gly/Asp variant is associated with human longevity.

Materials and Methods

Participants
Six hundred seventy-seven Caucasians (388 females and 289 males; mean age: 62.76 ± 26.01 years) from northern, central, or southern Italy volunteered for the study. In order
to assess the impact of the IRS2 gene polymorphism on longevity, on the basis of literature data (15), participants were categorized into two groups by splitting the whole sample at the age of 85: healthy people aged 85 years and younger were grouped under the denomination of “control people”; healthy people aged from 85 to 104 years were collected in the group of “long-lived people.”

For the enrollment of long-lived participants, a written permission with project explanation was submitted to large communities in the Italian regions in order to obtain a demographic list of participants born between 1908 and 1924. All participants were contacted at home or in their institution and examined by trained physicians. Control participants were selected from Italian geriatric medicine, internal medicine, or geriatric rehabilitation structures in regimen of Day Hospital, ordinary admission or outpatient office. After a clear explanation of the aims and the potential risk of the study, all participants gave informed consent to participate in the study, which was approved by the Ethical Committee at the University of Naples.

All participants were normotensive and had liver, kidney, and thyroid function tests within normal range. According to American Diabetes Association criteria (16), all participants were affected neither by diabetes nor by impaired fasting glucose. No participant used drugs affecting insulin secretion and/or action or plasma lipid levels. Participants with cancer, obesity, and major cardiovascular or endocrine diseases were excluded.

Analytical Methods
The participants were categorized as long lived (LL; >85 years old) or controls (C; <85 years old). Anthropometric determinations were measured as previously reported (17). Blood samples were collected in the morning after the participants had been fasting for at least 8 hours. Plasma glucose levels were determined by the glucose oxidase method (Beckman Glucose Autoanalyzer; Beckman Instruments, Fullerton, CA). Commercial enzymatic tests were used to determine serum total and high-density lipoprotein (HDL) cholesterol and triglycerides levels (Roche Diagnostics, GmbH, Mannheim, Germany). After centrifugation, plasma insulin concentrations were determined by enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden).

Genotyping
A total of 677 individuals were genotyped for Gly1057Asp-IRS2 locus variability (Cluster Report number: rs1805097). Genomic DNA was obtained from blood lymphocytes collected into ethylenediaminetetraacetic acid–containing tubes using a commercial DNA extraction kit (Illustra; GE Healthcare, Uppsala, Sweden). Genotyping was carried out by polymerase chain reaction (PCR)–restriction fragment length polymorphism. Details of primer sequences and experimental protocol are available upon request.

Statistical Analysis
The difference in genotype frequency was analyzed by chi-square test. For investigating difference in allele frequency, Fisher’s test was used. The chi-square test was used to compare the expected genotypic frequencies based on the Hardy–Weinberg equilibrium with the actual frequencies observed in the long-lived and control groups separately. Insulin resistance (Homeostasis Model Assessment [HOMA]-IR), insulin sensitivity (HOMA IS), and β-cell function (HOMA-B cell) were calculated by the HOMA2 calculator v2.2 (www.dtu.ox.ac.uk/homa). To approximate normal distributions, plasma insulin, HOMA-IR, and HOMA-B cell were logarithmically transformed for use in all calculations and back-transformed for result presentations. All metabolic parameters were presented as mean ± SD. Analysis of variance with Scheffe’s test was used for comparing the mean body mass index (BMI), HOMA2%B, and HOMA2 IR between control and long-lived male and female participants. A logistic regression analysis was used to test the association of IRS2 gene polymorphism with longevity (being LL vs C) independently of multiple covariates. Furthermore, in order to estimate the odds ratio, for different IRS2 genotypes, of reaching the extreme old age independently of multiple covariates (sex, HOMA-IR, HOMA%B, BMI, cholesterol, triglycerides, and HDL), participants were subdivided by age into 10 percentiles and used as ordinate dependent variables in multinomial regression with a logit link function. Such analysis expands the general linear model analysis so that the dependent variable is linearly related to the factors and covariates via a specified link function. The cumulative logit, \( f(x) = \ln(x/(1 − x)) \), applied to the cumulative probability of each category of the response. The odds ratio, 95% Wald confidence intervals, and the \( p \) value with the Bonferroni correction were calculated using SPSS.

Results
Our experimental population included 677 people aged between 16 and 104 years—289 males and 388 females—with metabolic characteristics in the normal range (Table 1). Long-lived participants displayed significantly lower BMI than controls (Figure 1A). Similar to IRS\(^{+/−} \) mice (2), long-lived participants displayed greater insulin sensitivity and reduced β-cell function compared with the controls (Figure 1B and C). The allele and genotype frequencies of the Gly1057-Asp IRS2 gene polymorphism in our experimental population were determined by PCR–restriction fragment length polymorphism.

In order to assess the potential effect of population stratification, we compared genotype and allele frequency distributions of IRS2 gene variants between the three geographically different Italian regions. Indeed, no differences in allele and genotype frequency distributions of IRS2 gene polymorphism between northern, central, or southern Italy were found (data not shown). The allele and genotype
frequencies observed were almost similar to those reported in previous studies among Caucasians.

In the whole population, homozygous IRS2 Asp/Asp participants were more represented among LL versus C participants (16.7% vs 12.0%; \( p = .04 \); Table 2). The association between IRS2 gene polymorphism with longevity (being LL) was independent of sex, HOMA-IR, HOMA%B, BMI, plasma cholesterol, triglycerides, and HDL levels (odds ratio: 2.07, 95% CI = 1.38–3.12; \( p = .001 \)).

Categorizing participants into percentiles by age, an ordinal logistic regression analysis was performed to determine whether the IRS2 Asp/Asp, IRS2 Gly/Asp, or the most common IRS2 Gly/Gly genotype was associated with extreme longevity (≥90 percentile, 96–104 years). As expected, women were more likely to be associated with the oldest participants, whereas a high BMI or HOMA2%B reduced significantly the odds of reaching the 90 percentile (Table 3). However, controlling for these covariates, the odds of reaching extreme old age was increased 2-fold for IRS2 Asp/Asp participants and 1.5-fold for IRS2 Gly/Asp participants (Table 3).

### Discussion

Our study supports the hypothesis that the Gly1057Asp variant of IRS2 is associated with human longevity. Participants with one or two IRS2 Asp alleles displayed a greater chance of living between 96 and 104 years of age. These results are consistent with experiments in mice showing that reduced IRS2 signaling increases life span up to 20% (5).

Even though our findings provide novel and intriguing evidence for the involvement of the IRS2 in the control of aging and longevity also in humans, the functional effects of the structural variation of the IRS2 due to the gene polymorphism remain unclear, and mechanism relating such variation with circulating insulin levels and longevity has to be established. However, the finding that IRS2 Asp/Asp together with obesity is associated with polycystic ovarian syndrome, reduced beta-cell function, and/or diabetes in certain populations (6,8–11) suggests that IRS2 Asp might have reduced function that exacerbates the effects of obesity. Without an underlying cause of obesity to promote

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**Table 1. Clinical Characteristics of Study Population (n = 677)**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 470)</th>
<th>Long Lived (n = 207)</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>289/388</td>
<td>239/231</td>
<td>—</td>
</tr>
<tr>
<td>Age (y)</td>
<td>62 ± 26</td>
<td>48 ± 15</td>
<td>.001</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.5 ± 3.2</td>
<td>25.1 ± 2.9</td>
<td>.001</td>
</tr>
<tr>
<td>Glucose (mmol/dL)</td>
<td>4.8 ± 0.9</td>
<td>4.9 ± 0.8</td>
<td>.001</td>
</tr>
<tr>
<td>Insulin (mU/mL)</td>
<td>10.6 ± 4.4</td>
<td>11.6 ± 4.2</td>
<td>.001</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>107 ± 45</td>
<td>108 ± 44</td>
<td>.540</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>198 ± 42</td>
<td>201 ± 41</td>
<td>.020</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>51.2 ± 10.6</td>
<td>49.6 ± 8.4</td>
<td>.001</td>
</tr>
</tbody>
</table>

**Notes:** BMI = body mass index; HDL = high-density lipoprotein.

*\( \chi^2 = 41.86; df = 1 \) \( p<0.05 \). All data presented are mean ± SD.
insulin resistance, IRS2<sup>Asp</sup> people might act like the IRS2<sup>+/−</sup> mice that have improved glucose tolerance and reduced β-cell function and live 20% longer than control mice.

As people age, compensatory hyperinsulinemia maintains euglycemia by opposing the effects of insulin resistance (13). Hyperinsulinemia/insulin resistance has been found implicated in the development of many diseases, such as hypertension, dyslipidemia, obesity, and atherosclerosis strongly affecting morbidity, disability, and mortality among the elderly participant (18,19). However, chronic hyperinsulinemia might also affect life span directly by slowly damaging brain function (18,19). Interestingly, in humans, genetically reduced insulin signaling is associated with a lower risk of cognitive impairment and increased survival beyond 85 years (20). Moreover, brain-specific deletion of one or both IRS2 alleles extends mouse life span about 18% (5). These findings suggest that consequences of the local action of insulin within the brain include effects on aging and longevity. We hypothesize that, like brain-specific IRS2 knockout mice, the IRS2<sup>Asp</sup> variant might protect the aging brain from the negative effects of hyperinsulinemia that ordinarily develop with overweight and advancing age by attenuating, also in humans, IRS2 signaling. In mice, it will be important to compare the phenotype of Thr1049Asp knock-in mouse variant (homologous to the human Gly1057Asp variant) to determine whether IRS2<sup>Asp</sup> also extends mouse life span.

Work with lower metazoans—mainly Caenorhabditis elegans and Drosophila—shows that diminished insulin-like signaling can extend the healthy life span. Humans aged 85–90 years and older display increased insulin sensitivity—centenarians are particularly insulin sensitive (14). Thus, reduced intensity and duration of the insulin signal—expression of an efficient insulin signaling—appears to be essential for healthy human aging. IRS2<sup>Asp</sup> signaling might have a similar effect to increase life span—at least when obesity or other causes of insulin resistance are absent—by attenuating insulin signaling through the IRS2 branch of the pathway.

As a whole, the animal data and the results of our study allow us to speculate that the IRS2 signaling cascade—possibly in the brain—integrates the effects of peripheral nutrient homeostasis with life span. Consistent with this hypothesis, calorie restriction, weight loss, and moderate daily exercise are the best studied strategies to reduce insulin levels and increase insulin sensitivity, improve metabolic regulation, and extend longevity (21,22). Our results reveal a starting point to understand the best ways to coordinate nutrient homeostasis and insulin signaling with strategies that consistently promote longevity and healthy aging (23).

Further studies will be necessary for replicating our finding in an independent larger population group with sufficient power before the association between IRS2 gene polymorphism and longevity can be regarded as proven. Furthermore, studies of interactions may be useful after basic replication is complete. Interaction with a different genetic and/or environmental background may, in fact, differently modulate the effect of a given gene in different populations.

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**References**


**Table 2.** Allele and Genotype Distributions of the IRS2 Gene Variant in Control (mean age = 48 ± 15 years, age range = 16–83 years) and Long Lived (mean age = 96 ± 4 years, age range = 85–104 years)

<table>
<thead>
<tr>
<th>Gene Variant</th>
<th>Control (n = 418)</th>
<th>Long Lived (n = 144)</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gly/Gly</td>
<td>46.1</td>
<td>34.7</td>
<td>χ² = 6.16; p = .04</td>
</tr>
<tr>
<td>Gly/Asp</td>
<td>41.9</td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>Asp/Asp</td>
<td>12.0</td>
<td>16.7</td>
<td></td>
</tr>
<tr>
<td>Gly + participants</td>
<td>53.8</td>
<td>65.3</td>
<td>Fischer’s test: p = .01</td>
</tr>
</tbody>
</table>

**Table 3.** Multinomial Regression of Age in Percentiles as the Ordinal Dependent Variable and Cumulative Logit as the Link Function With Genotype and Sex as Categorical Factors and HOMA2 IR, HOMA2% B, and BMI and the Serum Concentration of Cholesterol, Triglycerides, and HDL at Various Metabolic Parameters as Numerical Covariates (n = 677, male/female = 289/388, age range: 16–104 years)

<table>
<thead>
<tr>
<th>Parameter*</th>
<th>Odds Ratio†</th>
<th>Lower Confidence Interval</th>
<th>Upper Confidence Interval</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype IRS2&lt;sup&gt;Asp&lt;/sup&gt;/Asp</td>
<td>2.03</td>
<td>1.39</td>
<td>2.99</td>
<td>.0003</td>
</tr>
<tr>
<td>IRS2&lt;sup&gt;Gly/Gly&lt;/sup&gt;</td>
<td>1.49</td>
<td>1.15</td>
<td>1.94</td>
<td>.003</td>
</tr>
<tr>
<td>IRS2&lt;sup&gt;Asp/Gly&lt;/sup&gt;</td>
<td>1.49</td>
<td>1.16</td>
<td>1.91</td>
<td>.002</td>
</tr>
<tr>
<td>Sex Female</td>
<td>1.49</td>
<td>1.16</td>
<td>1.91</td>
<td>.002</td>
</tr>
<tr>
<td>Sex Male</td>
<td>1.49</td>
<td>1.16</td>
<td>1.91</td>
<td>.002</td>
</tr>
<tr>
<td>HOMA2 IR</td>
<td>0.80</td>
<td>0.64</td>
<td>1.00</td>
<td>.051</td>
</tr>
<tr>
<td>%B</td>
<td>0.998</td>
<td>0.995</td>
<td>1.000</td>
<td>.034</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>0.998</td>
<td>0.994</td>
<td>1.001</td>
<td>.15</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>1.000</td>
<td>0.997</td>
<td>1.003</td>
<td>.88</td>
</tr>
<tr>
<td>HDL</td>
<td>1.005</td>
<td>0.993</td>
<td>1.017</td>
<td>.44</td>
</tr>
</tbody>
</table>

**Notes:** BMI = body mass index; HDL = high-density lipoprotein; HOMO = homeostasis model assessment; IRS2 = insulin receptor substrate-2.

*Dependent variable is age (percentile).
†Odds ratio of reaching the 90% percentile for age (>96 years).
‡Set to 1 as the reference.


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