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ORIGINAL ARTICLE

Insulin-like growth factor 1 receptor polymorphism rs2229765 and circulating interleukin-6 level affect male longevity in a population-based prospective study (Treviso Longeva– TRELONG)

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Insulin-like growth factor 1 (IGF-1) signaling modulation has been associated with increased lifespan in model organisms, while high levels of circulating interleukin-6 (IL-6) are a marker of disability and mortality. In the prospective, population-based "Treviso Longeva"– TRELONG Study from Italy (*n* = 668, age range 70–105.5 years at baseline, followed for seven years) we investigated the effects of survival on the IGF-1 receptor (*IGF-1R*) gene polymorphism *rs2229765*, the *IL-6* gene promoter polymorphism *rs1800795*, and plasma concentrations of IGF-1 and IL-6, alone or in combination. We found a sex-dependent effect for the *IGF-1R rs2229765* polymorphism, as male carriers of the homozygous A/A genotype survived longer, while the *IL-6 rs1800795* genotype did not influence overall or sex-specific longevity. Higher IL-6 levels were more detrimental for survival among males than females, while IGF-1 had no dose–response effect. These findings sustain the hypothesis that sex-specific longevity relies on detectable differences in genetic and biochemical parameters between males and females.

Keywords: IGF-1, IL-6, aging, longevity, inflammation, male genetics

Introduction

Genetics and the environment influence human longevity. Several works have addressed the role of genetics in exceptional longevity [1–4], while the influence of genetics on longevity is supported in twin-based studies where the individual genome accounted for approximately 25% of lifespan [5,6].

The biochemical basis of longevity has been explored in model organisms like *Caenorhabditis elegans*, *Drosophila* and the mouse, leading to the identification of key cell pathways (oxidative stress response, DNA repair, inflammation and energy metabolism) related to successful aging [7,8]. The insulin/insulin like-growth factor 1 (IGF-1) pathway and the pro-inflammatory cytokine network seem to have an important role in modulating longevity [9–11].

Insulin/IGF-1 signaling (IIS) triggers intracellular downstream transcription factors as a consequence of a cascade starting from the membrane receptors of insulin and IGF-1 [12]. Biochemical or genetic attenuation of IIS has been associated with increased lifespan, probably due to slow cell growth rate and metabolism [13–15]. In humans, circulating IGF-1 decreases with age and is also modified by sex hormones [16–18]. A synonymous polymorphism (*rs2229765*) on the IGF-1 receptor gene (*IGF-1R)* [GenBank:NM_000875] consisting of G to A transition at nucleotide 3174 leading to the amino acid change Glu->Glu at position 1043 (E1043E) [GenBank:NP_000876], has been positively associated with longevity in the Italian population [19].

Inflammation has a significant and dual role in aging physiology, both as an acute inflammatory response that is beneficial for survival, but also as a chronic inflammatory state that often parallels neurodegenerative disorders,

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autoimmunity and cancer [20,21]. A key pro-inflammatory molecule is interleukin-6 (IL-6) whose circulating level in humans tends to rise over time [22]. IL-6 transcription rate is slowed by the single nucleotide polymorphism (SNP) *rs1800795(C/T)* located in the IL-6 gene promoter region [GenBank: NM_000600] [23]. The role of *rs1800795* in longevity has been explored in the Italian population, with controversial results [24–27]. However, a higher IL-6 circulating level is almost invariably a negative predictor of survival [22–28].

We assessed the influence of *IGF-1R rs2229765, IL-6 rs1800795,* circulating IGF-1 and IL-6 on survival curves in a sample of elderly people from Treviso (Italy) who were followed for seven years from baseline (TRELONG Study), with particular attention to sex-specific patterns.

Methods

Population sample recruitment and ethics

The TRELONG study has been described in detail elsewhere [29]. In brief, it started in 2003 in the municipality of Treviso. Participants were systematically sampled from the list of residents at the Registry Office of Treviso, based on an initial plan to include 100 participants per sex and 10-year age group; selection criteria included 125 women and 125 men aged 70–79 years and everyone over 100 years old. Of the 670 eligible, 668 participated (99.7% response rate): 311 men and 357 women, aged 70 years and older (mean age 84 ± 8 years). An interviewer-administered questionnaire and a blood sample were collected at participants' homes. Baseline characteristics of this study population and methodological details have been published previously, and form the basis for these analyses. The elderly sample was followed for seven years beginning in 2003; the dates of death were collected. Those who survived were assessed at the age they were on June 20th, 2009. The participants were evaluated using biologic, clinical and socioeconomic measures, with blood samples and a structured interview.

The study protocol was approved by the Ethics Committee of the National Institute on Research and Care of the Elderly (INRCA, Italy). Written informed consent was obtained from each participant or from a legally responsible person.

Blood sampling, rs2229765 and rs1800795 genotyping

Blood samples (about 30 mL) were collected by venipuncture. One part was centrifuged to isolate leukocytes to be used for genomic DNA (gDNA) extraction, using a semi-automated nucleic acid extractor (AB6100, Applied Biosystems, CA, USA). Another portion was centrifuged at 2000 rpm for 10 min at 4°C (with sodium EDTA as the anticoagulant) to separate the plasma, which was stored at−80°C until needed. Consent for blood collection was obtained from 590 of the 668 participants and 587 plasma samples were successfully prepared.

The *rs2229765* and *rs1800795* genotypes were assessed as previously described [30,31].

Plasma IGF-1 and IL-6 assays

Plasma IGF-1 was assayed by sandwich-type enzyme-linked immunosorbent assay (ELISA) (Diagnostic System Laboratories, Inc, Webster, TX, USA), according to the manufacturer's instructions. The kit sensitivity was 10 ng/mL and intra-assay %CV was <10%. Plasma IL-6 was measured by another sandwich-type ELISA (Ultra Sensitive ELISA Kit, Biosource, Camarillo, CA, USA), according to the manufacturer's instructions. The kit sensitivity was 0.10 pg/mL and intra-assay %CV was between 5 and 10%. Each plasma sample was tested in duplicate.

Statistics

The curves reported in Figures 1 and 2 were plotted by the Kaplan-Meier method. Survival curves were plotted separately by sex and by IL-6 or IGF-1 level. Odds ratios and 95% confidence intervals (CI) were calculated using sex-stratified Cox proportional hazard models, adjusted for age. Multivariate regression analysis was done considering mortality as a dependent outcome. Participants who died within the first three years were excluded from the analyses to control for reverse causality bias. Statistics were obtained by using the "survival" package of R software. Results were considered significant at $p < 0.05$, using two-tailed tests of significance.

Results

Effects of rs2229765 and rs1800795 on survival

The age and sex of the TRELONG study population are summarized in Table I. The sample included individuals from 70 years of age and older (six were ultracentenarians). The male-to-female ratio was 0.87. The survival curves were plotted by sex and stratifying for *IGF1R rs2229765* genotype [Figure 1A and 1B]. No significant effect comes to light. For men, the odds ratio [and 95% confidence interval (CI)], corrected for age, and considering the A/A genotype as reference were: A/G 2.29 [0.54–9.59], $p = 0.25$; G/G 2.54 [0.57–11.27], $p = 0.22$. For women, the odds ratio [and 95% CI], corrected for age, and considering the A/A genotype as reference were: A/G 0.81 [0.38–1.73], $p = 0.60$; G/G 0.95 [0.43–2.10], $p = 0.91$. However, a multivariate regression analysis including several confounders (age, CVD, VC, total cholesterol, diabetes, cancer and smoking) (Table II) indicated that the *rs2229765* G/G genotype significantly increased the hazard ratio in comparison to the A/A genotype, suggesting a positive role of the latter on survival.

For *IL-6 rs1800795*, we did not find any influence on survival [Figure 1C–1D]. In fact, for men the odds ratio [and 95% CI], corrected for age, and considering the C/C genotype as reference were C/G 0.77 [0.29–2.03], $p = 0.60$; G/G 0.69 [0.25–1.87], $p = 0.47$, while for women the odds ratio [and 95% CI], corrected for age, and considering the C/C genotype as reference were C/G 2.3 [0.55–10.14], $p = 0.24$; G/G 2.2 [0.52–9.40], $p = 0.28$. A multivariate regression confirmed the lack of influence on survival (data not shown).

Effects of circulating IGF-1 and IL-6 on survival

We have previously reported that circulating levels of IL-6 rise with age and correlate with disability and mortality [32]. We have now extended this observation, considering a possible sex-specific pattern [Figure 2A and 2B]. IL-6 had a more pronounced effect on survival in men than in women. In fact, in men IL-6 levels in the second quartile were already marginally significant in increasing mortality risk ($p = 0.06$) and became a clearly deleterious predictor of survival from the third quartile onwards. In men, the calculated odds ratio [and 95% confidence interval (CI)] for the single curves, corrected for age and considering the 1st quartile as reference, were the following: 2nd quartile: 2.3 [0.95–5.71], $p = 0.06$; 3rd quartile: 3.5 [1.5–8.1], $p = 0.003$, 4th quartile: 3.0 [1.2–7.1], $p = 0.012$. For women, the odds ratio [and 95% CI] for the single curves, corrected for age and considering the 1st quartile as reference were as follows: 2nd quartile, 0.98 [0.4–2.1], $p = 0.96$; 3rd quartile 0.8 [0.3–2.0], $p = 0.65$; 4th quartile 1.1 [0.5–2.4], $p = 0.80$.

Multivariate analysis, reported in Table II, showed that IL-6 levels in the fourth quartile were associated with a significant

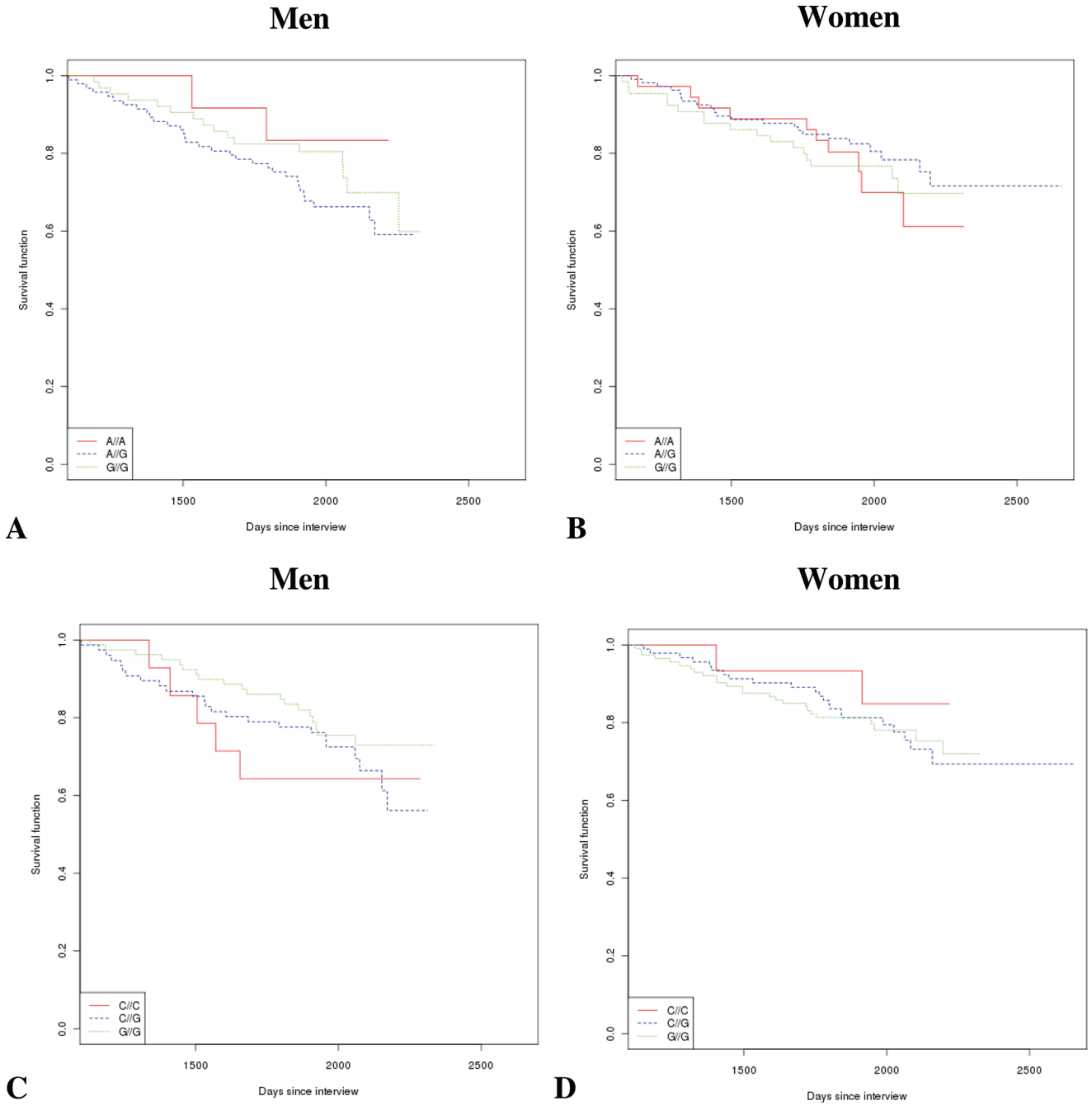

Figure 1. Survival analysis according to sex and genotype. (A) Survival for males according to *IGF-1R rs2229765* (G/A) polymorphic site. (B) Survival for females according to *IGF-1R rs2229765* (G/A) genotype. (C) Survival for males grouped according to *IL-6 rs1800795* (C/G) polymorphic site. (D) Survival for females stratified for IL-6 *rs1800795 (C/G)* polymorphism.

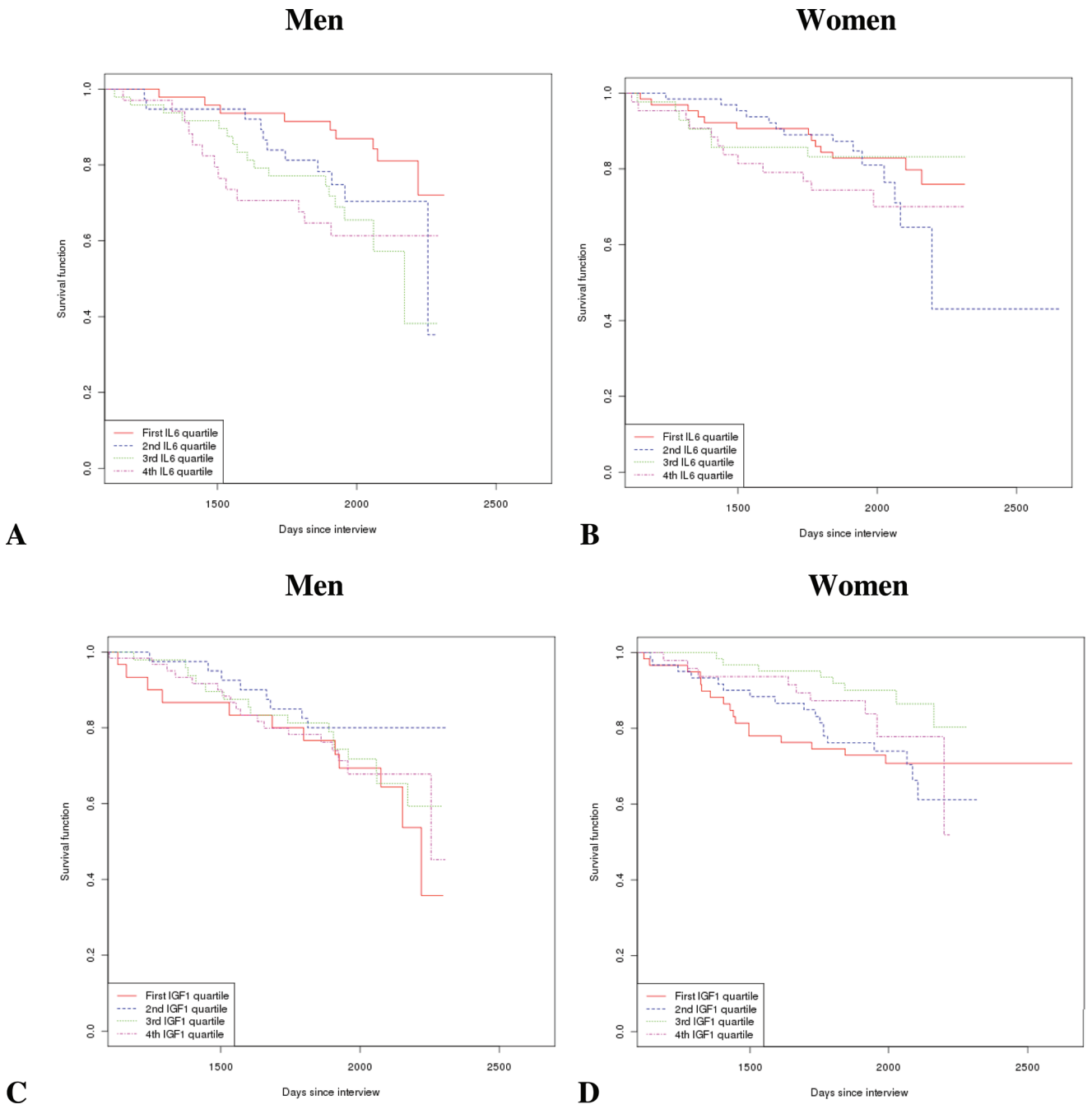

Figure 2. Survival curves on the basis of sex and circulating levels of IL-6 or IGF-1. (A) Survival in men stratified by IL-6 level in plasma (in quartiles). (B) Similar analysis in females. (C) Survival analysis according to sex and circulating levels of IGF-1 (in quartiles) in men only. No significant difference came to light considering the 1st quartile as reference and correcting for age. (D) The same analysis as in (C), for women. The individual functions showed no significant differences.

Table I. Distribution by age of the elderly subjects in the TRELONG study. For a full study description see Gallucci et al. [29].

Age (years) (no. of individuals)	Sex (male:female)
70–74 (129)	61:68
75–79 (132)	72:60
80–84 (126)	64:62
85–89 (73)	29:44
90–94 (168)	74:94
95–99 (34)	10:24
100–106 (6)	1:5
Whole study (668)	311:357

increase in mortality, although the association was attenuated in women.

We have already shown an age-related decrease in IGF-1 levels that was more significant in males [30]. However, the survival curves of participants were similar when plotted according to IGF-1 level and sex [Figure 2C and 2D]. These data were confirmed in the multivariate regression analysis (data not shown).

We also checked a possible combined effect of IL-6 and IGF-1 on survival. First, we divided our population according to combinations of IL-6 and IGF-1 (low or high level,

Table II. Regression to assess the effect on survival of *IGF-1R rs2229765* polymorphic site (a) and of circulating IL-6 (b). CVD: cardiovascular disease (including myocardial infarction, ischemic cardiopathy, peripheral vasculopathy and chronic heart failure); VC: vascular cerebral disease (including stroke and cerebral vasculopathies). C: regression coefficient; HR: hazard ratio; St. er: Standard error.

a. IGF-1 rs2229765

MEN	C	HR	St. er	*t*-Value	*p*-Value
Age	0.1	1.11	0.02	4.4	<0.01
CVD	1.14	3.14	0.37	3.06	<0.01
VC	−0.01	0.99	0	-1.99	0.05
Cholesterol (total)	0.01	1.01	0.39	0.02	0.99
Diabetes	0.85	2.33	0.34	2.46	0.01
Cancer	0.01	1.01	0.32	0.04	0.97
Smoking	1.35	3.88	1.03	1.32	0.19
IGF1, A/G (ref: A/A)	1.42	4.14	1.04	1.37	0.17
IGF1, G/G (ref: A/A)	0.1	1.11	0.02	4.4	<0.01
WOMEN					
Age	0.11	1.12	0.02	4.58	<0.01
CVD	0.28	1.32	0.38	0.72	0.47
VC	0.08	1.08	0.42	0.19	0.85
Cholesterol (total)	−0.01	0.99	0	−1.99	0.05
Diabetes	−0.35	0.71	0.39	−0.88	0.38
Cancer	0.04	1.04	0.45	0.09	0.93
Smoking	0.06	1.07	0.38	0.17	0.87
IGF1, A/G (ref: A/A)	0.41	1.51	0.38	1.07	0.29
IGF1, G/G (ref: A/A)	−0.36	0.7	0.45	−0.79	0.43

b. circulating IL-6

MEN	C	HR	St. er	t-Value	p-Value
Age	0.09	1.1	0.02	4.05	<0.01
CVD	0.35	1.42	0.33	1.06	0.29
VC	0.67	1.95	0.41	1.64	0.1
Cholesterol (total)	−0.01	0.99	0	−1.49	0.14
Diabetes	−0.18	0.83	0.38	−0.49	0.63
Cancer	0.64	1.9	0.36	1.8	0.07
Smoking	−0.01	0.99	0.32	−0.03	0.98
IL-6, 2nd quartile (vs. 1st)	0.51	1.67	0.47	1.09	0.28
IL-6, 3rd quartile (vs.1st)	1.02	2.77	0.44	2.31	0.02
IL-6, 4th quartile (vs. 1st)	0.91	2.49	0.46	1.98	0.05
WOMEN					
Age	0.13	1.14	0.03	4.84	<0.01
CVD	0.05	1.05	0.41	0.12	0.9
VC	0.06	1.06	0.42	0.14	0.89
Cholesterol (total)	0	1	0	−0.92	0.36
Diabetes	−0.49	0.61	0.41	−1.19	0.24
Cancer	−0.16	0.85	0.54	−0.30	0.76
Smoking	0.25	1.28	0.39	0.64	0.52
IL-6, 2nd quartile (vs. 1st)	−0.02	0.98	0.41	−0.06	0.96
IL-6, 3rd quartile (vs. 1st)	−0.20	0.82	0.49	−0.40	0.69
IL-6, 4th quartile (vs. 1st)	0.13	1.14	0.03	4.84	<0.01

expressed in quartiles). We obtained four groups whose main demographic and clinical features are summarized in Table III. A high level of IL-6 was associated with increased disability and co-morbidity, while high IGF-1 was associated with increased percentage of cancer in association to low IL-6 only. We considered the group with low IL-6 and high IGF-1 (that had an apparent reduced rate of mortality) as reference and plotted a survival curve for the whole population classified according to levels of IL-6 and IGF-1 [Figure 3A]. The statistical analysis demonstrated a global effect (log-rank test $p < 0.001$), even if the individual curves had no significant difference (data not shown). However, the group with reduced performance in survival was the opposite of the reference (high IL-6 and low IGF-1). We also tried to analyze survival according to a combination of IL-6 and IGF-1 level and sex [Figure 3C and 3D]. The situation was similar to the entire population, with a global significant effect (log-rank test $p < 0.001$ both for males and females); for men and women, the group with the apparent reduced longevity had a high level of IL-6 and a reduced level of IGF-1, even if the statistic analysis for the single curve did not evidence any significance.

Discussion

According to the Italian National Institute of Statistics (ISTAT), the province of Treviso has one of the longest life spans in Italy, particularly for women (http://demo.istat.it/) [33]. Therefore, the TRELONG study is uniquely positioned to investigate genetic and environmental factors in longevity. We first considered the genetic variability due to *rs2229765* polymorphism of the *IGF-1R* gene. The survival curves suggested the homozygous A-allele genotype was a positive predictor of survival in men, in comparison to the genotype G/G; this was confirmed by multivariate regression analysis. We previously reported that in a cross-sectional analysis in the TRELONG population, there was a significantly ($p = 0.04$) higher A-allele frequency in males over 85 [30]. Another study in Italy by Bonafé et al. reported an increase of the same *rs2229765* A-allele in men and women over 85 years of age [19]. To the best of our knowledge, our prospective study that supports the pro-surviving effect of the A/A homozygous genotype, at least in men, is the only one available in the literature.

The drop in circulating IGF-1 with age did not correlate with survival in our analysis. However, the actual pro-survival significance of a low level of IGF-1 is debated [34,35]. While low IGF-1 reduction might counteract cancer, it correlates with an increased risk of all-cause mortality and cardiovascular disease [36,37]. Moreover, a low level alone might be inadequate as a predictor of survival, as reported by Maggio et al. in another Italian population of elderly men, where IGF-1 as well as also testosterone and dehydroepiandrosterone sulfate (DHEA-S) was evaluated; they found a cumulative negative effect on survival for these anabolic hormones [38]. Our data on IGF-1 may be inconclusive due to the lack of multiple hormone assessment.

We also addressed the contribution of an inflammatory pathway to longevity. Since aging involves chronic increases in pro-inflammatory molecules, an aspect that is particularly evident for men and is partly under genetic control [39], we looked for a sex-specific pattern in biochemical or genetic elements related to IL-6. Our prospective approach found that the IL-6 plasma level influenced survival more in men, and no genetic contribution was found from the polymorphism *rs1800795*, though we cannot exclude that a fuller genetic investigation of IL-6 promoter region might uncover additional genetic variability correlating with male or female longevity.

Table III. Classification of the TRELONG population according to circulating levels of IGF-1 and IL-6. *p*-value was calculated from Chi-square test for binary variables and ANOVA for continuous variables. CVD: cardiovascular disease (including myocardial infarction, ischemic cardiopathy, peripheral vasculopathy and chronic heart failure); VC: vascular cerebral disease (including stroke and cerebral vasculopathies); CCI: Charlson's comorbidity index.

	High IGF-1 and low IL-6	Low IGF-1 and low IL-6	High IGF-1 and high IL-6	Low IGF-1 and high IL-6	Overall	*p*-Value
Age (mean)	82.68	86.13	85.81	88.48	84.13	**0.000**
Women (%)	52.30	55.67	48.04	71.88	53.44	0.116
BMI (mean)	25.14	24.09	24.76	24.23	24.77	0.127
VC(%)	14.80	20.62	18.63	9.38	15.57	0.339
CVD (%)	24.67	24.74	32.35	21.88	25.6	0.426
Cholesterol (total, mean)	223.50	210.70	206.30	180.50	213.8	**0.000**
Diabetes (%)	15.46	18.56	17.65	15.62	15.57	0.884
Cancer (%)	20.07	8.25	11.76	12.50	16.92	**0.021**
ADL (mean)	5.32	4.88	4.57	4.13	5.002	**0.000**
CCI (mean)	5.59	5.85	6.16	6.25	5.766	**0.005**
Smoking (%)	41.58	42.71	41.58	25.00	41.17	0.293

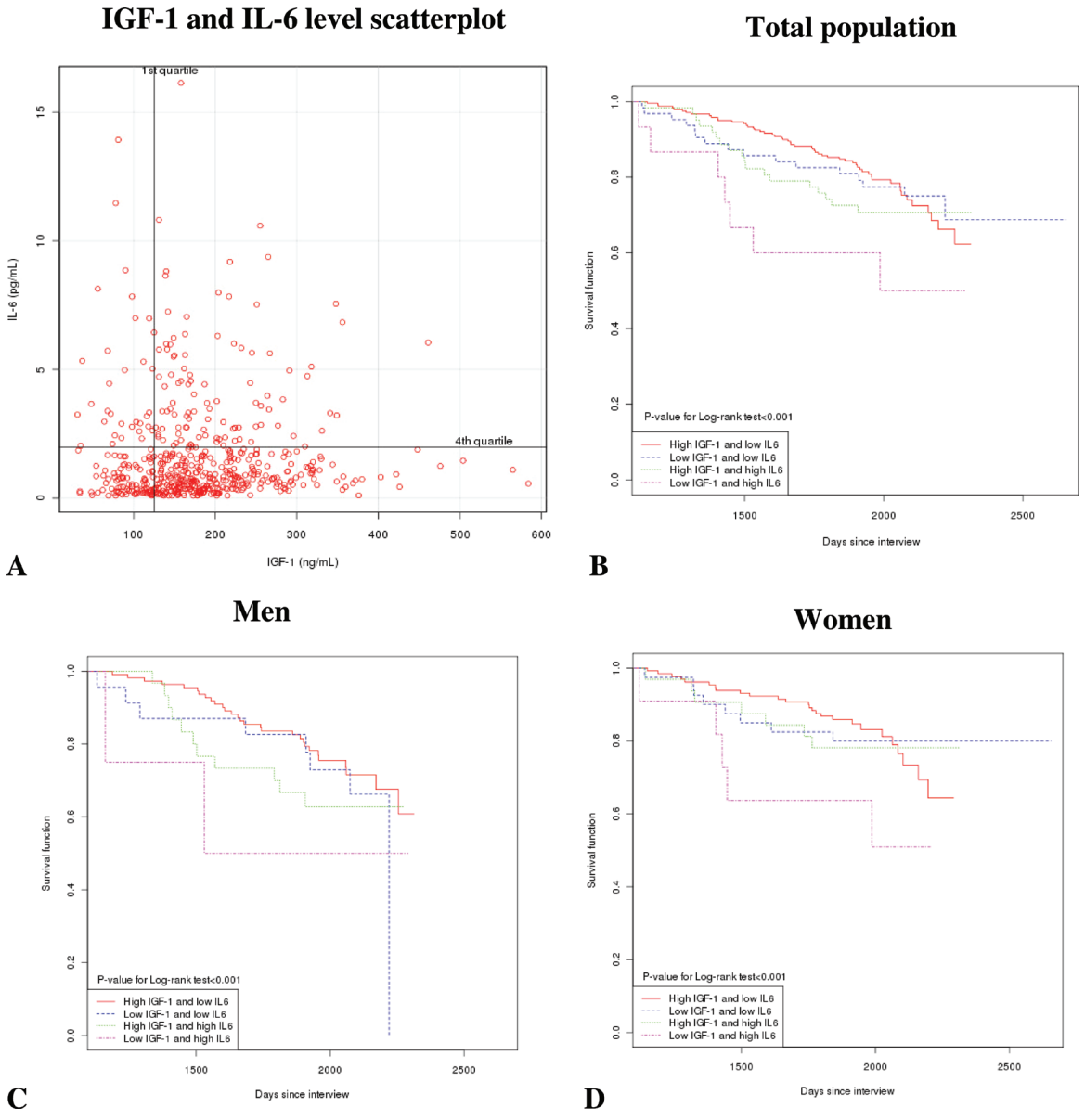

Figure 3. Assessment of combined effect of IGF-1 and IL-6 on survival. (A) The TRELONG population was divided according to low or high levels of circulating IL-6 and IGF-1 (divided into quartiles). (B) Survival curve of the entire population classified according to high/low level of IL-6 and IGF-1 (C) Survival curve as in (B) for males only; (D) Survival curve for females classified according to IL-6 and IGF-1 assessed level. For the survival analysis, the curve of high IGF-1 and low IL-6 was considered as reference.

Our combined analysis, looking at the same time the effect of different levels of IL-6 and IGF-1, has confirmed that high levels of IL-6 are detrimental for survival, while in combination with high IGF-1, low IL-6 seems to be associated with increased longevity. The power of this analysis is reduced by limited sample size, even if the conclusion is in good agreement with Cappola et al. [40], showing a deleterious synergistic effect of high IL-6 and low IGF-1 in survival and disability in older women.

In summary, our seven-year follow-up assessing survival in a general elderly population from the province of Treviso, Italy, confirmed that males and females do differ in genetic and biochemical parameters correlating with longevity and suggested a possible combined effect of these two factors on survival.

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