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Chapter 4

***IL7R* gene expression network associates with human familial longevity**

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Abstract

The level of expression of the interleukin 7 receptor (*IL7R*) gene in blood was recently found to be associated with familial longevity and healthy ageing. The *IL7R* is crucial for T cell development and important for immune competence. To further investigate the *IL7R* pathway in ageing we identified the closest interacting genes to construct an *IL7R* gene network that consisted of *IL7R* and six interacting genes: *IL2RG*, *IL7*, *TSLP*, *CRLF2*, *JAK1* and *JAK3*. This network was explored for association with chronological age, familial longevity and immune-related diseases (type 2 diabetes, COPD and rheumatoid arthritis) in 87 nonagenarians, 337 of their middle-aged offspring and 321 middle-aged controls from the Leiden Longevity Study (LLS). We observed that expression level of the *IL7R* gene network as a whole was significantly different between the nonagenarians and middle aged controls ($P=4.6 \times 10^{-4}$) being driven by a significantly lower level of expression of *IL7R*, *IL2RG* and *IL7* in the elderly. After correction for multiple testing and blood cell subsets, only *IL7R* was found to be associated with familial longevity exhibiting lower levels in middle aged offspring of nonagenarian siblings than in age matched controls ($P=0.006$). Higher *IL7R* gene expression in these groups associated with a higher prevalence of immune-related disease ($P=0.001$). Together our results indicate a positive correlation of *IL7R* gene expression with biological age. Paradoxically however a higher *IL7R* gene expression level associated with better prospective survival both in nonagenarians ($HR=0.63$, $P=0.037$) and the middle aged individuals ($HR=0.33$, $P=1.9 \times 10^{-4}$). We conclude that the *IL7R* network reflected by gene expression levels in blood may influence the biological age and health status of elderly individuals.

Introduction

Ageing is considered to be the consequence of an accumulation of physiological changes over time eventually increasing the mortality risk. Although ageing is the major risk factor for reduced health status and the most common human diseases of Western societies, treatment of elderly patients may be improved by understanding their biological age, the rate at which a person ages, and health status better than just by indicating their chronological age.

Many potential biomarkers of biological age have been suggested and are being tested for their association with chronological age, diseases and prospective mortality (1). In a search for new transcriptomic biomarkers of

ageing we found that the expression in blood of 1853 genes was associated with chronological age, 244 of which were associated with familial longevity, being differentially expressed between middle aged offspring of nonagenarian siblings and controls in the Leiden Longevity Study (LLS). Of the latter group of genes, a low expression level of the interleukin 7 (IL7) receptor (*IL7R*) associates with low disease prevalence among these offspring (2). The IL7 receptor is important for the body's innate and adaptive immune responses, and plays a role in regulating development, differentiation and survival of T cells (3;4). The IL7R is required for IL7 signaling, which in mice was shown to be crucial for early T cell development, as well as for homeostasis of naïve and memory CD8⁺ T cells (5;6). Previously, we observed that the offspring of nonagenarian siblings avoid the usual age-related reduction of percentages and numbers of naïve T cells in the periphery (7). Reduction of proinflammatory IL7 signaling may contribute to this better retention of naïve T cells and may thereby influence the biological age and health status of elderly individuals, given that possessing a fuller naïve T cell repertoire would be expected to protect better against new pathogens.

To explore whether the expression profiles of other genes in the close vicinity of *IL7R* may exhibit even better ageing biomarker properties, we first identified the six interaction partners of *IL7R* using the STRING protein-protein-interaction database. Next, gene expression levels of all seven genes in the *IL7R* network were determined in 87 nonagenarians also having a nonagenarian sibling, 337 of their offspring and 321 controls of the LLS. We tested whether the level of expression of the *IL7R* network genes associated with chronological age by comparing the nonagenarians with the middle-aged controls. Differential expression in this comparison may be explained by the age difference between these groups, early environmental factors, or by the longevity trait in these nonagenarians which is not present in controls. To further investigate whether the expression of the age-associated genes also associates with biological age and disease prevalence, we compared the controls with the similarly aged offspring of the nonagenarians, representing individuals with a reduced biological age marked by lower prevalence of common age-related disease and beneficial metabolic profiles (8;9). Additionally, we explored whether the expression of the *IL7R* interaction partners is associated with common immune-related diseases in middle-aged individuals. Finally, we performed survival analysis in the nonagenarians to determine the relationship between expression of these genes and mortality. Because whole blood consists of multiple cell types, all associations have been adjusted for the distribution of the cell types.

Results

IL7R network

To identify proteins interacting with the IL7 receptor, we searched for known and predicted protein-protein interactions using the freely-accessible STRING 9.0 data base (<http://string-db.org/>) in May 2012. Interactions based on text mining were excluded, while predicted interactions based on experimental data with the highest confidence (score > 0.900) were taken into account. This approach resulted in an IL7R network that consists of IL7R and the following six interacting proteins: IL2RG, IL7, TSLP, CRLF2, JAK1 and JAK3 (Figure 1).

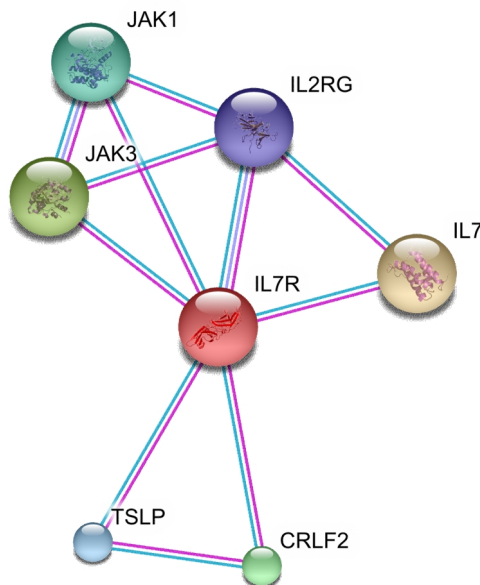


Figure 1. IL7R STRING network

Gene expression in whole blood

We measured gene expression levels for each of the seven genes using RT-qPCR on whole blood samples from 87 nonagenarians, 337 of their middle-aged offspring and 321 middle-aged controls (Table 1). To test for associations between expression of genes in the IL7R network and chronological age, we compared long-lived individuals to the middle-aged controls for differential expression of the total gene set of the *IL7R* network using a global test (10;11). We observed that the expression level of the

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IL7R gene set as a whole was significantly different between the groups (P-value=4.6 x 10⁻⁴).

Table 1. Subject characteristics

	Nonagenarians	Offspring	Controls
Number	87	337	321
Mean Age	94.3	61.3	61.2
Age Range	89.0 - 101.7	33.6 - 78.3	32.4 - 81.4
Women (%)	47 (54.0%)	143 (42.4%)	175 (54.5%)
N T2D (%)	NA	17 (5.0%)	26 (8.1%)
N COPD (%)	NA	19 (5.6%)	10 (3.1%)
N RA (%)	NA	3 (0.9%)	2 (0.6%)

N T2D: number of known patients with Type 2 Diabetes, N COPD: number of known patients with chronic obstructive pulmonary disease, N RA: number of known patients with rheumatoid arthritis, NA: data not available.

To investigate which genes were primarily responsible for the association of the *IL7R* network with age, we tested single gene expression levels using linear regression of the seven genes in the *IL7R* network (Table 2). After Bonferroni correction for multiple testing, three genes showed significant differential expression with at least a 5% difference between nonagenarians and middle aged controls, namely *IL7R*, *IL2RG* and *IL7*. We observed that expression of the IL receptor complex/ligand genes *IL7R*, *IL2RG*, *IL7*, *TSLP* and *CRLF2* were all lower in nonagenarians, while expression of *JAK1* and *JAK3* was increased.

Table 2. Gene expression of nonagenarians compared to controls by linear regression analysis.

Gene	Coef	FC	P	Bonferroni
IL7R	-0.40	0.76	<10-6	0.000
IL2RG	-2.09	0.23	0.007	0.049
IL7	-0.81	0.57	<10-6	0.000
TSLP	-0.01	0.99	0.013	0.091
CRLF2	-0.15	0.90	0.040	0.280
JAK1	0.21	1.16	0.046	0.322
JAK3	1.68	3.20	0.009	0.063

Coef: coefficient from linear regression model. FC: fold change, above one indicated higher expression in long-lived individuals. p: raw p value from linear regression model. Bonferroni: p value after adjustment for multiple testing (n=7) by the Bonferroni method. Genes significantly differentially expressed with at least 5% are depicted in bold.

Because differential expression in these comparisons may be explained by the age difference between the two groups, cohort effects, or by the

longevity trait in these families which is not present in controls, we investigated whether the differences in expression of the seven IL7R network genes was characteristic for these long-lived families, exhibiting a lower biological age, and not just a marker for chronological age. Therefore, we compared the expression in the middle-aged offspring of nonagenarians to their partners serving as controls matched for age and adult lifestyle and environmental conditions. Expression of all the IL7R complex/ligands *IL7R*, *IL2RG*, *IL7*, and *TSLP* and of *JAK3* was found to be lower in offspring of nonagenarians than in controls. Of these, *IL7R* remained significant after Bonferroni correction for multiple testing (Table 3), indicating its association with familial longevity and biological age. Similar results were obtained after adjustment for blood cell counts (Supplementary Table S1). Expression of *JAK1* was higher in offspring as compared to controls. The direction of the differential gene expression was the same as for nonagenarians, except for *JAK3* for which the expression was increased in nonagenarians and decreased in their offspring relative to controls.

Table 3. Gene expression of offspring from nonagenarians compared to controls by linear regression analysis.

Gene	Coef	FC	P	Bonferroni
IL7R	-0.18	0.89	0.001	0.006
IL2RG	-0.72	0.61	0.563	1.000
IL7	-0.39	0.77	0.050	0.350
TSLP	-0.01	0.99	0.140	0.980
CRLF2	-0.04	0.97	0.742	1.000
JAK1	0.08	1.05	0.557	1.000
JAK3	-0.30	0.81	0.650	1.000

Coef: coefficient from linear regression model. FC: fold change, above one indicated higher expression in offspring from long-lived individuals. p: raw p value from linear regression model. Bonferroni: p value after adjustment for multiple testing (n=7) by the Bonferroni method. Genes significantly differentially expressed with at least 5% are depicted in bold.

Relation of IL7R gene expression in whole blood with membrane-bound IL7R protein in PBMCs

Since decreased *IL7R* gene expression associates with familial longevity already in middle-age, the question arises whether this reflects soluble or membrane-bound IL7R protein, also known as cd127. We were able to investigate membrane-bound cd127 levels in PBMCs of 71 offspring and 73 controls. We observed no correlation between *IL7R* gene expression in whole blood and cd127 levels in PBMCs of the same individuals (Table 4) and no difference in cd127 between offspring and controls (mean level offspring=38.1, mean level controls=37.5, p-value=0.91). It is therefore less

likely that the difference in gene expression levels reflect differences in levels of membrane-bound cd127.

Table 4. Correlation of *IL7R* gene expression and cd127 expression in offspring from nonagenarians and controls.

Subset	Offspring (n = 53)		Controls (n = 53)	
	Cor	p	Cor	p
PBMC	0.161	0.250	-0.171	0.221
Lymphocytes	0.169	0.226	-0.135	0.336
T cells	0.174	0.212	-0.159	0.256
Non-T cells	0.152	0.278	-0.130	0.353
CD4+ cells	0.169	0.227	-0.151	0.281
CD8+ cells	0.167	0.231	-0.135	0.336

Cor: Pearson Correlation. p: raw p value from Pearson Correlation.

Association of *IL7R* gene expression with immune-related disease

Because *IL7R* and IL7 signaling have been implicated in the etiology of immune-related disease (12-15), the observed differences in gene expression with biological age might also be associated with different immune-related disease prevalence between the groups (Table 1). We investigated expression levels of *IL7R* relative to disease status for type 2 diabetes (T2D), chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis (RA) in the offspring of long-lived individuals and controls. Table 5 shows that higher *IL7R* expression is associated with a higher prevalence of immune-related diseases. However, the difference in *IL7R* expression between offspring and controls did not change when adjusted for prevalence of T2D, COPD and RA (Supplementary Table S2). Thus, the *IL7R* expression levels in blood associate with immune-related disease on the one hand and familial longevity on the other, independent of these immune-related diseases.

Table 5. Association of *IL7R* gene expression with immune-related diseases in middle aged subjects

Gene	T2D (n=43)		COPD (n=29)		RA (n=5)		Sumscore (n=70)	
	Coef	p	Coef	p	Coef	P	Coef	p
IL7R	0.22	0.029	0.35	9.9E-05	-0.09	0.391	0.21	0.001

Total: total prevalence of T2D, COPD or RA. Coef: coefficient from linear regression model. FC: fold change, above one indicated positive association between gene expression and disease prevalence (patient = 1, control = 0). p: raw p value from linear regression model. Number of known patients is mentioned in table, the total number of offspring and controls is 658. Significant associations are depicted in bold.

Association of IL7R gene expression with mortality

Many markers indicating health status in middle age (such as blood pressure) associate also with mortality at higher ages, albeit not always in the expected direction (16). We examined whether the *IL7R* gene expression level is associated with prospective mortality in the subset of LLS participants for which we have measured expression levels. We performed a survival analysis using a Cox-proportional hazard model for low versus high gene expression levels in 81 nonagenarians (Model 1) and 619 offspring and partners (Model 2). The hazard ratio among the nonagenarians was 0.63 (95%CI 0.40-0.97) with a P-value of 0.037, and the hazard ratio among the middle aged individuals was 0.33 (95%CI 0.18-0.59) with a P-value of 1.91×10^{-4} . Unexpectedly, higher *IL7R* gene expression levels in blood associated with better survival in both age groups.

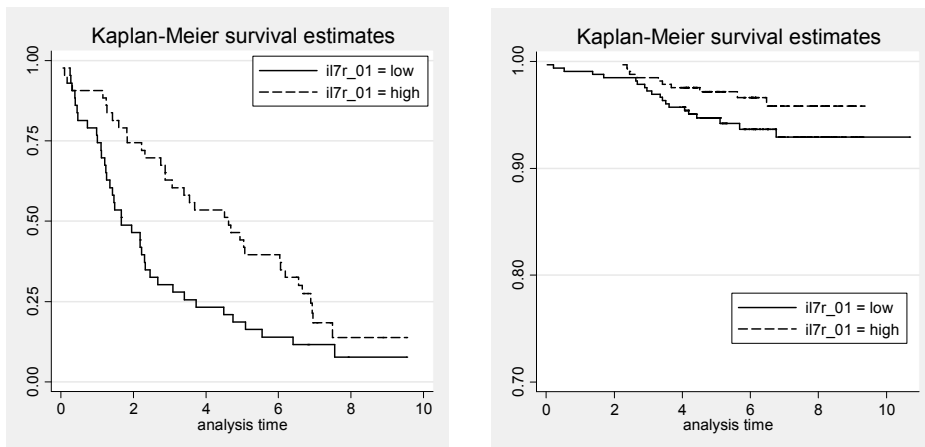


Figure 2. Kaplan-Meier curve for high (dotted line) and low (solid line) *IL7R* gene expression in nonagenarians (left panel) and middle aged subjects (right panel).

Discussion

Gene expression levels in blood of the *IL7* geneset as a whole correlates negatively with chronological age. The expression of the *IL7R* gene in particular was found to be indicative for biological age and immune-related diseases. Relative to controls, reduced *IL7R* expression characterized members of longevity families, both middle aged offspring and nonagenarian siblings, whereas increased expression associated with prevalence of immune-related diseases in these groups (T2D and COPD). Paradoxically, however, higher *IL7R* gene expression levels associated

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with better survival in a prospective analysis of both the nonagenarians and middle aged subjects. The mechanism underlying these observations requires further investigation, and especially whether regulation of *IL7R* gene expression has a causal role as determinant of biological ageing.

The notion that low *IL7R* expression levels are beneficial for reaching old age healthily corresponds with previous observations that autoimmune disease patients express increased levels of the IL receptor/ligand complex genes (12;13;15) and that antagonizing IL7 or IL7R may offer possible treatment (14;15). An increase in systemic inflammation has generally been reported with increasing age, so-called “inflamm-ageing” (17). Long-lived individuals such as LLS nonagenarians can be seen as slow or healthy agers who do not show the commonly observed age-related characteristics of “immunosenescence” and display relatively low levels of pro-inflammatory markers (7). Our results may suggest that nonagenarian members of long-lived families have more efficient IL7 signaling since they seem to require less compensation because of their remaining naïve T cell population, resulting in greater reserve capacity to cope with infections in old age. On the other hand, very low IL7R signaling has been observed in severe combined immunodeficiency (SCID) (18) and in HIV infection (19;20). We conclude that, besides the troublesome effect of the absence of IL7 signaling, a somewhat lower baseline level of *IL7R* gene expression, and potentially IL7 signaling, may contribute to healthy aging.

Despite these observations, the expression of the *IL7R* gene network was negatively correlated with age suggesting that low expression levels correlate with decreasing health, consistent with our observations that low *IL7R* gene expression associates with higher prospective mortality. Clearly this finding is inconsistent with the observation that in the same group of subjects low *IL7R* gene expression level is associated with familial longevity and decreased disease risk. A similar paradox has been found in PBMCs, where a lower frequency of naïve T cells (and a higher frequency of differentiated T cells) was associated with better survival in elderly individuals while a higher frequency was found in offspring of nonagenarians compared to the controls suggesting an association with lower biological age (7;21). This might be interpreted as follows: if the individual is able to counter the reduction of *IL7R* gene expression with age by keeping the level of differentiated T cells high as memory cells to control disease, this may be associated with healthy old age.

Contributing to the paradox may be the fact that there are two forms of IL7R exerting different functions, membrane-bound and soluble IL7R.

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Membrane-bound IL7R may transduce IL7 signaling, while soluble IL7R may represent a negative compensatory mechanism regulating IL7 signaling (20;22;23). Because we used the recommended Taqman assay for the measurement of *IL7R* gene expression that cannot distinguish between the *IL7R* splice forms, we are unable to interpret how IL7 signaling is affected by the gene expression changes. Since *IL7R* gene expression levels and membrane-bound IL7R protein did not correlate in offspring or controls, and also the protein levels did not show a difference between the two groups, our results might suggest that the decrease in *IL7R* gene expression reflects mainly a decrease in the mRNA coding for soluble IL7R, resulting in more efficient IL7 signaling.

We showed that *IL7R* gene expression in blood is associated with immune-related disease. Previous meta-analysis and GWAS studies showed that genetic variation in the *IL7R* gene is associated with ulcerative colitis (24), multiple sclerosis (25-27), primary biliary cirrhosis (28), and type 1 diabetes (29). These results may suggest that genetic variation in and the expression of the *IL7R* gene is involved in auto-immune and chronic inflammation disease. Hence optimal response of the immune system may contribute to human longevity.

Further evidence that IL7 signaling may contribute to biological ageing and longevity is that it is closely connected to mTOR signaling, a pathway known for its effects on lifespan in animal models and which is now also implicated in human aging and longevity (30;31). Several studies in mice provided evidence for the connection between IL7 and mTOR signaling. IL7 induces phosphorylation of the mTOR complex 1 (mTORC1) downstream targets S6 and 4EBP1, an effect antagonized by the mTOR inhibitor rapamycin. The reverse may occur as well: rapamycin inhibits proliferation and induces apoptosis of pre-B acute lymphoblastic leukemia (ALL), effects which are abrogated by IL7 (32). Functional studies validated *IL7R* as a FoxO1 target gene (33). Acute deletion of FoxO1 induced a rapid and profound downregulation of IL7R expression associated with a significant reduction of *IL7R* mRNA (34). Also, cytokine (including IL7) stimulation induces FoxO1 phosphorylation and decreased transcription of target genes (34). In B cells, it has been shown that the mTOR complex 2 (mTORC2) suppresses *IL7R* gene expression by regulating FoxO1 phosphorylation (35). Taken together, decreased IL7 levels and decreased mTORC1 activity seem to go hand-in-hand, further implying that decreased IL7 levels are beneficial because decreased mTORC1 increases lifespan. The same is true for decreased IL7R and increased mTORC2, the latter we have recently shown to be associated with human longevity (31). Gene

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expression levels of several of the mTOR-related genes are indeed positively and significantly correlated with *IL7R* expression in the LLS, which included *RPTOR*, *FOXO1* and *MTOR*. Considering the clear connections with IL7 signaling and similar findings on the level of gene expression variations, mTOR signaling might also be involved in “inflamm-ageing” as part of its lifespan-regulating effect.

In a previous study within the LLS families Dekker et al (36) found in the cultured skin fibroblasts of the offspring of the nonagenarians more apoptotic activity and less senescence than in those of controls. Flow cytometric analysis in HIV-infected individuals demonstrated that CD8 cells expressing high levels of *IL7R* also expressed slightly higher levels of anti-apoptotic markers, whereas nearly all apoptotic cells had low levels of *IL7R* (37). Our finding of decreased *IL7R* expression in members of long-lived families fits well with these previous results.

In conclusion, an overall lower expression level in blood of genes belonging to the IL7R network was found to be associated with higher chronological age. Yet, low *IL7R* gene expression was significantly associated with familial longevity in middle-age independent of blood cell counts and high expression level with prevalence of T2D, COPD and RA. Intriguingly nonetheless, higher *IL7R* gene expression associates with better prospective survival. The level of expression of the *IL7R* gene in the blood is a very promising marker for healthy ageing in long lived families, although further research is required to understand how *IL7R* gene regulation contributes to biological ageing.

Materials & Methods

Study population

Leiden Longevity Study

The individuals investigated in this study are participants of the Leiden Longevity Study. The families participating in this study have at least two siblings with a minimum age for men of 89 years and for women of 91 years (38). The offspring of these long-lived individuals, who have an increased chance of becoming long-lived (30% reduced standardized mortality rate), were also included. In addition, the partners of the offspring were included as population controls of similar age and environmental exposures as the offspring, and as a young control group for the nonagenarian siblings. Blood samples were taken from all the participants. The Leiden Longevity Study was approved by the Medical Ethical

Committee of Leiden University Medical Centre and all participants gave written informed consent.

Sample collection and RNA preparation

87 non-related long-lived siblings, 337 offspring and 321 partners belonging to 281 nuclear families were selected for the current study (Table 1 and Supplementary Table S5). These samples were randomly selected, but in such a way that age and gender were balanced between the groups and age range was as large as possible. Only individuals without outlying cell counts (beyond 3 SDs below or above the standard error of the mean) were included. This subpopulation is representative for the whole LLS regarding disease prevalence and parameters involved in metabolic syndrome (Supplementary Table S5) (9;39). From these non-fasted individuals, peripheral blood was harvested using PAXgeneTM tubes (Qiagen, Venlo, The Netherlands). The tubes were frozen and kept at -20°C for ~3-5 years. After thawing at room temperature for at least 2 hours, total RNA was extracted from the approximately 2.5 ml of peripheral blood in each tube following the manufacturer's recommended protocol (PAXgene Blood RNA Kit Handbook, Qiagen, Venlo, The Netherlands). The quality and integrity of the total RNA was evaluated on the 2100 Bioanalyzer (Agilent Technologies, Amstelveen, The Netherlands) and the concentration was measured using a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Quality criteria included a 28S/18S ratio as measured by the Bioanalyzer of at least 1.2, and a total RNA yield of at least 3 µg.

RT-qPCR

For all 7 IL7R network genes the suggested Taqman[®] assay (Applied Biosystems) was selected. Reverse transcription was performed with total RNA from blood of in total 745 samples, which passed QC using the First Strand cDNA Synthesis Kit according to the manufacturer's protocol (Roche Applied Science). cDNA was amplified using the DNA Engine Tetrad[®] 2 Peltier Thermal Cycler (Bio-Rad). qPCR was then performed with the Taqman[®] method using the BiomarkTM 48.48 and 96.96 Dynamic Arrays (Fluidigm). Relative gene expression of the BioMarkTM Array data were calculated by using the 2- $\Delta\Delta C_t$ method, in which C_t indicates cycle threshold, the fractional cycle number where the fluorescent signal reaches the detection threshold (40). YKT6 was used as internal control and commercially available human total reference RNA (Clontech Laboratories, Mountain View, CA, USA) as reference RNA.

Geneset analysis of gene expression data

The Globaltest methodology was designed to determine whether the common expression pattern of genes within a pre-defined set is significantly related to clinical outcome (10;41). A generalized linear model is used to estimate a "Q-statistic" for each gene set, which describes the correlation between gene expression profiles, X , and clinical outcomes, Y . The Q-statistic for a gene set is the average of the Q-statistics for each gene in the set. The Globaltest method was used to perform geneset analysis comparing two groups of individuals (either nonagenarians vs. controls or offspring vs. controls) including age (in offspring vs. controls only) and gender and their interaction as covariates. Global test package in R (10) has been used to perform analyses.

Single gene analysis of gene expression data

Differences in expression level between nonagenarians, their offspring and the partners of their offspring were assessed using linear regression. In these analyses, expression level was the dependent variable and the two groups of individuals (either nonagenarians vs. controls or offspring vs. controls) were included in the model as a categorical variable together with age (in offspring vs. controls only), gender and their interaction as covariates. To take into account dependencies within sibships, robust standard errors were used, i.e. the variance was computed from the between family variation. P-values were also based on these robust standard errors. Analyses were performed using the software package STATA/SE 11.0 (DPC Software, StataCorp 2009).

Blood cell subtypes

To further investigate the candidate genes, their expression level was associated with several blood cell counts. In the whole blood samples of the participants the following cell subtypes were counted using the automated Siemens ADVIA 1200 system (SMSD, Tarrytown, NY) in the Leiden Medical Diagnostics Center: leukocytes, thrombocytes, neutrophils, lymphocytes, monocytes, basophils and eosinophils. Next, both the comparison between nonagenarians and controls and between offspring and controls were adjusted for each of these cell counts separately.

CD127 staining

Cryopreserved peripheral blood mononuclear cells (PBMC) were thawed and treated with human immunoglobulin (GAMUNEX; TalecrisBiotherapeutics) and ethidiummonoazide (EMA, Invitrogen) to block Fc receptors and stain dead cells followed by an indirect staining for CD3 using an OKT3 supernatant and a pacific-orange-cojugated anti-mouse IgG (Invitrogen). After blocking unbound secondary antibodies with mouse serum (Chemicon, Millipore), cells were surface-stained with CD4-Pacifid Blue, CD127-Alexa Fluor 647 (BioLegend, San Diego, USA) and CD8-PerCP (BD Biosciences, Heidelberg, Germany). Cells were measured immediately using an LSR-II (BD).

For data analysis, EMA+ dead cells were excluded and lymphocytes were gated using an FSC-vs.-SSC dot plot based on their size and granularity. T-cells and non-T-cells were characterized as CD3+ and CD3- cells within the lymphocyte gate. In the CD3+ gate, CD4 and CD8 cells were characterized in an CD4-vs.-CD8 dot plot as CD4+CD8- and CD4-CD8+ cells, respectively. The mean fluorescence intensity (MFI) of Alexa-Fluor647 (CD127) was determined on total lymphocytes, CD3-, CD3+, CD4+ and CD8+ cells. To standardize for fluctuation of the instrument over the measurement period of a few weeks, MFI of each studied population was standardized against the MFI of an unstained control PBMC for each experimental day, by dividing the MFI of each population by that of the cells in the lymphocyte gate of the unstained control. Flow cytometry data analysis was performed using the FlowJo software (Tristar, San Diego, USA).

Association of gene expression with immune-related diseases

Information on medical history was requested from the participants' general practitioners. Gene expression of *IL7R* and *IL7* was associated with prevalence of T2D, COPD and RA and the sum score of these diseases. The sum score indicates the number of patients with T2D, COPD or RA or a combination thereof. Next, the comparison between offspring and controls for *IL7R* and *IL7* expression association with longevity was adjusted for the prevalence of these diseases, using the linear regression model described above.

Prospective survival analysis

Prospective analysis of IL7R-related genes was performed with 81 nonagenarians (Model 1), 313 offspring and 306 controls (Model 2). After a mean follow-up time of 7.40 (nonagenarians) and 6.15 years (offspring and controls), 87.7% (n = 71, nonagenarians) and 4.5% (n = 28, offspring and controls) of the individuals had died. Mortality analyses were performed with STATA/SE 11.2 (StataCorp LP, TX, USA) using an age at inclusion, sex and white blood cell count-adjusted, left-truncated Cox proportional hazards model to adjust for late entry into the dataset according to age.

Model 1: $\lambda(t) \sim \lambda_0(t) \exp(\beta_1 * \text{age at inclusion} + \beta_2 * \text{sex} + \beta_3 * \text{lymphocyte count} + \beta_4 * \text{neutrophil count} + \beta_5 * \text{monocyte count} + \beta_6 * \text{eosinophil count} + \beta_7 * \text{basophil count} + \beta_8 * \text{Gene})$

Model 2: $\lambda(t) \sim \lambda_0(t) \exp(\beta_1 * \text{age at inclusion} + \beta_2 * \text{sex} + \beta_3 (\text{age} * \text{sex}) + \beta_4 * \text{group} + \beta_5 * \text{lymphocyte count} + \beta_6 * \text{neutrophil count} + \beta_7 * \text{monocyte count} + \beta_8 * \text{eosinophil count} + \beta_9 * \text{basophil count} + \beta_{10} * \text{Gene})$

Age at inclusion was coded in years, sex was coded as 1 (male) or 2 (female) and group was coded as 1 (control) or 2 (offspring). Robust standard errors were used to account for sibship relations.

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Supplementary information

Table S1. Linear regression results of gene expression of offspring from long-lived individuals compared to controls, adjusted for blood cell numbers.

Gene	No adjustment		Neutrophils		Lymphocytes		Monocytes		Eosinophils		Basophils	
	Coef	p	Coef	p	Coef	p	Coef	p	Coef	p	Coef	p
IL7R	-0.18	0.001	-0.16	0.003	-0.15	0.004	-0.17	0.001	-0.18	0.001	-0.17	0.001
IL2RG	-0.72	0.563	-0.34	0.787	-0.19	0.877	-0.19	0.882	-0.51	0.687	-0.52	0.681
IL7	-0.39	0.050	-0.38	0.049	-0.35	0.070	-0.44	0.032	-0.45	0.024	-0.44	0.030
TSLP	-0.01	0.140	-0.01	0.121	-0.01	0.134	-0.01	0.103	-0.01	0.112	-0.01	0.092
CRLF2	-0.04	0.742	-0.05	0.715	-0.04	0.765	-0.05	0.713	-0.06	0.651	-0.09	0.515
JAK1	0.08	0.557	0.08	0.530	0.08	0.541	0.15	0.245	0.10	0.471	0.08	0.530
JAK3	-0.30	0.650	-0.36	0.593	-0.37	0.593	-0.08	0.905	-0.30	0.668	-0.29	0.680

Coef: coefficient from linear regression model. FC: fold change, above one indicated higher expression in offspring from long-lived individuals. p: raw p value from linear regression model. Genes significantly differentially expressed with at least 5% are depicted in bold.

Table S2. Linear regression results of IL7R gene expression of offspring from long-lived individuals compared to controls, adjusted for prevalence of immune-related diseases.

Gene	No adjustment		Adjusted for T2D		Adjusted for COPD		Adjusted for RA		Adjusted for total	
	Coef	p	Coef	p	Coef	p	Coef	p	Coef	p
IL7R	-0.18	0.001	-0.17	0.001	-0.19	2.4E-4	-0.18	0.001	-0.18	0.001

Total: total prevalence of T2D, COPD or RA. Coef: coefficient from linear regression model. FC: fold change, above one indicated higher expression in offspring from long-lived individuals. p: raw p value from linear regression model. Genes significantly differentially expressed with at least 5% are depicted in bold.

